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STUDIES ON THE GAPEWORM *SYNGAMUS TRACHEA* (MONTAGU, 1811) IN ROBINS AND CHICKENS[1,2]

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Manter and Pinto (1928) described *Syngamus tenuispiculum* from the robin in Nebraska but indicated that possible variations in the chicken gapeworm (*Syngamus trachea*) might show that the robin gapeworm is also *S. trachea.* The studies reported below were intended to determine the relationship between the robin and chicken gapeworm.

Syngamus trachea has been reported from the robin by Walker (1886). Walker also pointed out that the larvae of *S. trachea* were present in earthworms, and showed that earthworms were not a necessary host but could produce the disease in chicks that fed upon them. Walker experimentally infected robins with gapeworms from the chicken by feeding three young robins in the nest several infected earthworms. On examination, two of the robins were found to harbor three or four gapeworms each. In another experiment a young robin just from the nest was fed a large number of larvae hatched in water. It was kept in a cage hanging under a tree and fed by the old bird. On examination, three fair sized syngami were found in the trachea. The experimental infections of robins—as done by Walker—were incidental to attempts to prove the importance of earthworms in the transmitting of gapeworms to chickens, and the experiments were not well enough controlled to be conclusive.

S. trachea is known from various wild birds in England and the starling apparently is of considerable importance in the spread of the disease among domestic poultry in that country. Lewis (1926) showed that starlings play an important rôle in the distribution of gapeworms in chickens, while Taylor (1928) contended that the eggs obtained from the gapeworm of starlings have only a low infectivity to young chickens, but

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[2] The present studies were made under the direction of Dr. H. W. Manter. The author is also indebted to Dr. Benjamin Schwartz of the U. S. Bureau of Animal Industry for sending specimens of *Eisenia foetida* infected with *Syngamus trachea* from the chicken.

"once an infection has been obtained the second generation enters the chick readily." Clapham (1935) showed quite conclusively—by using the indirect method of infection—that the starling strain of gapeworms is very infective to chickens. Rice (1929) transmitted a gapeworm indistinguishable from *S. trachea* from the rook (*Corvus frugilegris*) to the chicken.

Cram (1927) gave a list of 28 species of wild birds in which "gapeworms identical in structure with *Syngamus trachea* have been obtained." The record of *S. trachea* from the robin in America by Walker (1886) and Megnin's (1883) report of its occurrence in the swift were not included in her list. In addition to these hosts, *S. trachea* has also been reported from the sparrow, linnet, martin, kestrel, pigeon, and sandpiper (Campbell, 1935), and from Gambel's sparrow, water thrush, and junco (Cram, 1931). Cram noted the common occurrence of the parasite in wild birds in Alaska where it has not been reported from the chicken.

In this country, *S. trachea* has been reported as occurring naturally in turkey, chicken, robin, bobwhite quail, ruffed grouse, and pheasant. The possible rôle played by wild birds in the dissemination of gapeworms in the United States is unknown.

It is known that the life cycle of *S. trachea* can be completed with or without an earthworm but usually a higher percentage of infections as well as a larger number of worms are secured when infected earthworms are fed to experimental chicks. To Waite (1920) belongs the credit for establishing the fact that earthworms are an important factor in the transmission of gapeworms—as indicated by Walker. Clapham (1934) found more certain and more severe infections through the use of *Eisenia foetida* than when other species of earthworms were used.

Of 76 robins collected at Lincoln, Nebraska, 25 or about 33 per cent were infected with gapeworms. Usually only one pair of worms was present but one robin contained three pairs. The worms occurred all along the trachea but usually in the lower third.

MORPHOLOGY

The morphology of these worms from the robin may be briefly described as follows (measurements in millimeters):

Male: Length, 1.146 to 4.239 (average of 31 specimens, 2.631). Buccal capsule, 0.189 to 0.288 in external diameter (average 0.209), with cuticular rim. Esophagus, 0.503 to 0.706 (average 0.601). Dorsal ray cleft, each branch usually bidigitate, sometimes tridigitate (in one specimen, one branch was bidigitate, the other unforked); externo-dorsal ray rather short and thick; lateral rays about equal and close together; ventral rays similar. Spicules slender, usually subequal, rarely equal, 0.608 to 0.805 in length.

Female: Length, 6 to 14 (average of 31 specimens, 9.5); width, 0.320 to 0.735. Vulva 2.19 to 3.50 from the anterior end. Buccal capsule, 0.193 to 0.278 in external diameter (average, 0.235), with cuticular rim. Esophagus, 0.518 to 0.785 in length

(average 0.611). Number of teeth varying from seven to nine in both sexes, usually eight; tendency toward nine teeth in the female and seven in the male. Eggs average 0.090 by 0.047.

This gapeworm appears to differ from *S. merulae* in the presence of a buccal rim and different dorsal rays. There seem to be no morphological grounds for separating gapeworms of the robin and chicken, and *S. tenuispiculum* is considered a synonym of *S. trachea*.

INFECTION EXPERIMENTS

Methods: Gapeworms were removed from the trachea; the uteri dissected out, and as far as possible the gut and anterior end of the worms removed to keep the cultures clean. The eggs were placed in small dishes and incubated in a constant temperature box at a temperature ranging from 25° to 27° C. Most of the eggs were in the 4-, 8- or 16-cell stage when removed from the uterus. The cultures were kept relatively free of bacterial and fungoid growths by daily changes of water. On the 6th day the larva was clearly discernible through the eggshell. It was usually found that dissolution of the end "plugs" began on the 10th and 11th days. At the time of feeding, larvae had already hatched or could be clearly seen moving inside the shell. The species of earthworms used in the experiments were *Eisenia foetida* and *Helodrilus caliginosus*. These were kept in groups of 20 in flower pots containing soil that had been sterilized. At the end of 10 days the earthworms were washed and brushed and transferred to fresh sterilized soil before eggs were fed to them. The chicks used in the experiments were all incubator hatched and raised in the laboratory under controlled conditions.

In attempts to infect chickens directly with the infective stage of the robin gapeworm, the eggs were concentrated by drawing off as much of the water as possible. A drop of the culture was then placed on a slide and the infective eggs counted. A small quantity of flour was then added with the water until a small pellet containing the eggs was formed. This was fed to the chicks. By this method, Morgan and Clapham (1934) estimated that the size of the dose could be controlled to within an error of not more than 5 per cent. Feeding the earthworms the infective *Syngamus* eggs was accomplished by transferring the worms to dishes containing a small amount of soil, and washing infective eggs into the dishes. The earthworms remained in contact with the soil for four weeks or more before they were used in feeding experiments.

Exper. 1: Four one-day old chicks were fed the infective eggs and larvae of the robin gapeworm. Each chick received approximately 150 infective eggs and many recently hatched larvae. The chicks were killed one at a time at weekly intervals and examinations made of the lungs and trachea. None of the chicks revealed any indication of gapeworms.

Exper. 2: Each of four chickens four weeks old was fed three earthworms (*Helodrilus caliginosus*) from the soil seeded with the infective eggs of the robin gapeworm. The chicks were killed one at a time at intervals of one week from the time of the last feeding. No evidence of gapeworms was found.

Exper. 3: A nest of three very young robins was kept under close observation. They were each fed one earthworm (*Eisenia foetida*) containing the larvae of the chicken gapeworm. When the robins were about two weeks old they were removed to the laboratory. Two died shortly after capture. Through an oversight one individual was not examined. The other was autopsied but no gapeworms were found. The third robin was killed and examined two weeks after it was removed from the nest. No gapeworms were found.

Exper. 4: A robin just learning to fly was captured and fed several earthworms (*Helodrilus caliginosus*) from the soil seeded with the infective eggs of the robin gapeworm. Ten days after the robin was experimentally fed it was killed and examined. One small (♀ 7 mm) pair of gapeworms was recovered.

Exper. 5: Six one-week old chicks were fed earthworms (*Eisenia foetida*) from the soil seeded with the infective eggs of *S. trachea* from the robin. Each chick received one worm each, daily, for four days. One week later three of the chicks were killed and found uninfected. Two weeks after the chicks were experimentally fed the remaining three were killed. One chick harbored one large pair of gapeworms. No worms were found in the other two chicks.

The earthworms used in this experiment had been previously fed the infective larvae of the robin gapeworm in the laboratory. It was considered unlikely that the earthworms carried a natural infection of *Syngamus* since the gapeworm disease is rare or absent among poultry in this area. While the infection above seems to represent a cross from robin to chicken via *Eisenia foetida* it must be noted that the earthworms were collected in nature and it is not impossible that they contained the larvae of *S. trachea* from the chicken.

Exper. 6: In this experiment large doses of the infective eggs of the robin gapeworm were given to six one-day old chicks for a period of six consecutive days. The exact number was undetermined, but several thousand infective eggs were fed each chick. Three weeks after the first feeding, all of the chicks were destroyed and examinations were made of the lungs and trachea. Two of the chicks harbored one large pair of gapeworms each.

The number of chicks used in this experiment was small, but light infections were obtained in a third of the flock. It must be noted that the infective eggs used in this experiment differed from those of earlier

experiments since they had been in cold storage for six months before they were incubated, and increased infectivity of the eggs may have been due to this circumstance.

DISCUSSION

The above experiments indicate that the robin gapeworm cannot *easily* be transmitted to the chicken either by feeding the infective eggs, or by feeding earthworms (*Helodrilus caliginosus*) from the soil seeded with the infective eggs of *Syngamus* from the robin. No infections in young robins resulted from feeding earthworms (*Eisenia foetida*) containing the larvae of the chicken gapeworm. An infection in a robin occurred (*Exper. 4*) after feeding earthworms (*Helodrilus caliginosus*) from the soil seeded with the infective eggs of the robin gapeworm. This infection, however, might have originated before the young robin was captured. Exper. 6 seems to show definitely that robin gapeworm eggs when fed in large numbers can produce an infection with adult gapeworms in chickens. It seems doubtful if such cross-infections occur in nature.

SUMMARY

1. Morphologically the robin gapeworm could not be distinguished from *S. trachea* of domestic poultry. Therefore, *Syngamus tenuispiculum* Manter and Pinto, 1928, is considered to be a synonym of *Syngamus trachea*.

2. Several attempts to infect chickens with recently incubated eggs from the robin gapeworm were unsuccessful.

3. Earthworms (*Eisenia foetida*) fed incubated eggs of the robin gapeworm produced an infection in one of six chickens.

4. Eggs of the robin gapeworm kept in cold storage for six months, then incubated and fed in large numbers resulted in infections in two of six chickens.

5. Cross-infection experiments, therefore, confirm the identity of the robin and chicken gapeworms. However, because of the difficulty of cross-infection it is by no means certain that such transfers occur in nature.

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THE INTESTINAL PHASE OF THE RESISTANCE OF RABBITS TO THE LARVAE OF *TAENIA PISIFORMIS*

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In a previous report, the senior author (Leonard, 1940) presented data to show that in the case of infections with the larvae of *T. pisiformis* in the albino rabbit, an intense and accelerated tissue reaction results in the death of most of the parasites that succeed in reaching that organ. However, in immune animals, relatively few of the onchospheres fed actually reach the liver, judging from the number of typical cysticercal lesions which may be observed there following infection. There is a possibility that the larvae are destroyed in the tissues of the liver before they have time to produce an observable lesion, but the known facts suggest the hypothesis that the intestine, or intestinal wall of immune animals becomes a serious barrier to the migration of the onchospheres from the intestine to the liver. In order to test this hypothesis, the following experiment was devised.

EXPERIMENTAL PROCEDURE AND RESULTS

Ova of *T. pisiformis* were freed from the ripe proglottids and immersed in Tyrode's solution, to which had been added 1.0% trypsin, 0.5% dog bile by volume, and calcium carbonate to adjust the reaction to approximately pH 7.3. The ova hatched readily in this solution at 37 degrees C, and could be separated from most of the debris of the embryophore by gentle centrifugation. This method is similar to that used by Bullock et al (1934) in studies with *T. taeniaformis*.

Although Solomon (1934) reported that larvae of *T. pisiformis* "after being swallowed by the rabbit migrate from the stomach . . .," our findings fail to confirm this view. We have made repeated attempts to hatch the larvae in artificial gastric juice without success, even though a wide range of variations in the concentrations of both the enzyme and the acid was used. Onchospheres placed directly in the duodenum of the rabbit by operation hatched quickly, but our studies on this phase of the problem are as yet incomplete, and we are unable to state whether or not penetration occurs at the same level of the gut as hatching.

Ten normal albino rabbits were operated under ether anesthesia, and approximately 2000 artificially hatched onchospheres were injected into

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a branch of the portal vein. At the same time, five rabbits were selected from the original lot of fifteen as controls, and each was given 2000 *Taenia* ova per os. Rabbits of both groups were killed on the seventh day following this procedure, and the livers were preserved for study.

Fifteen rabbits similar in age and weight to the first group were passively immunized by the intravenous administration of 5 cc of immune serum per kilo of body weight. Twenty-four hours later, ten of these animals were operated, and each received 2000 hatched onchospheres via the mesenteric vein. The remaining five animals of this group were set aside as controls, and each was given 2000 *Taenia* ova per os. These fifteen animals were likewise sacrificed on the seventh day following infection, and livers preserved for study.

In the case of the normal animals, infection directly in the portal circulation by means of the hatched onchospheres was somewhat more successful than infection by the natural route. The number of cysticercal lesions observed on the livers of the ten operated animals varied from a minimum of 1167 lesions to a maximum of 1400 lesions, with an average number of approximately 1272 lesions per liver. In the control group which was infected by the natural route, the number of lesions varied around an average number of 956 lesions per liver, with a minimum of 788 lesions, and a maximum of 1108 lesions. The results obtained from the normal animals are summarized in Table 1.

TABLE 1.—*The number of liver lesions produced in normal rabbits by inoculating into the mesenteric vein 2000 artificially hatched onchospheres of* T. pisiformis, *contrasted with controls administered 2000* T. pisiformis *ova per os*

Onchospheres via mesenteric vein		Controls; ova per os	
Animal	Number of lesions	Animal	Number of lesions
233	1230	243	1007
234	1167	244	878
235	1256	245	998
236	1301	246	1108
237	1280	247	788
238	1244	Average	956
239	1260		
240	1400		
241	1384		
242	1211		
Average	1273		

The average number of lesions per liver in the case of the immunized animals that were infected via the mesenteric vein was slightly higher than in the case of non-immune animals similarly treated, but the difference is not significant when statistical analysis is applied to the figures. In these rabbits, the number of lesions varied from a minimum of 1190 lesions to a maximum of 1460 lesions, with an average number of approximately 1339 per liver. However, in the controls on this group, which were infected per os, the number of lesions observed on the livers was greatly

reduced; the average number was only 39 lesions per liver, with a minimum of 30 lesions, and a maximum of 47 lesions. Results obtained from the immune animals are summarized in Table 2.

TABLE 2.—*The number of liver lesions produced in immune rabbits by inoculating into the mesenteric vein 2000 artificially hatched onchospheres of* T. pisiformis, *contrasted with controls administered 2000* T. pisiformis *ova per os*

Onchospheres via mesenteric vein		Controls; ova per os	
Animal	Number of lesions	Animal	Number of lesions
387	1460	397	34
388	1288	398	47
389	1348	399	42
390	1401	400	30
391	1254	401	43
392	1397	Average	39
393	1305		
394	1387		
395	1190		
396	1366		
Average	1339		

DISCUSSION

At present, we have no data concerning the actual mechanism of the resistance factor which prevents the appearance of the great majority of the larvae in the livers of immune animals. It is not impossible, but it is improbable that the larvae are killed in the lumen of the intestine. Chandler (1935) showed that parasites in the lumen of the intestine which feed on the mucosa are not killed, but do have their nutrition interfered with so that they cannot grow and develop properly. Our studies indicate very rapid penetration of the intestine, which would make it improbable that the larvae feed until after invasion of the tissues. Therefore, the destruction of the larvae probably takes place after penetration of the mucosa, where death is due to some sort of tissue reaction. A further indication of this is the fact that Chandler (1936) showed that parenteral immunity extends to the walls of the intestine. Campbell (1936, 1938a, 1938b) showed that there are two types of immunity developed against the cysticerci of *T. taeniaformis* in rats and *T. pisiformis* in rabbits; an "early" form which brings about destruction of the parasites before encystment, and a "late" immune reaction which causes them to die after encystment. The antibodies causing "early" immunity are absorbable with fresh worm antigen and are stimulated by artificial immunization. The antibodies causing "late" immunity are not absorbable with fresh worm antigen, and do not follow artificial immunization. The "late immunity" antibodies are probably formed in response to some metabolic products of the growing worms and are comparable with the antibodies that interfere with the nutrition of *Nippostrongylus,* and which form precipitates about the mouth, anus, and excretory pore of the larvae of this worm (Sarles and Taliaferro, 1936). The destruction of the larvae

in these experiments before their encystment in the liver would seem to be due to "early immunity" antibodies described by Campbell, while the inhibition of growth and eventual death of the larvae after encystment are undoubtedly due to this author's "late immunity" antibodies.

SUMMARY

From the data presented, the following conclusions may be drawn:

1. In normal animals, the intestinal tract may possibly be a slight barrier to the migration of *T. pisiformis* larvae, since a greater average number of lesions was observed on the liver of animals infected via the portal circulation than in animals which were infected per os. However, this apparent natural resistance is probably not of great significance, since the loss of many ova may be accounted for by purely mechanical factors, such as intestinal peristalsis.

2. In the case of immunized rabbits, the intestinal tract is in some way a formidable barrier to the migration of *T. pisiformis* larvae, a conclusion which is supported by the significant difference between the average number of 1339 cysticercal lesions per liver in the case of animals infected via the portal circulation, and average number of 39 lesions per liver in the case of animals infected per os.

3. The resistance of immunized rabbits to infection with the larvae of *T. pisiformis* exists in two well-defined phases; an intestinal phase which is very significant, and a parenteral phase which disposes of most of the larvae that succeed in passing the intestinal barrier.

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BLOOD PARASITES OF BIRDS OF THE DISTRICT OF COLUMBIA AND PATUXENT RESEARCH REFUGE VICINITY

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Recently a number of surveys of blood parasites of birds have been made in different parts of this country by different workers, the general purpose of which was to further the knowledge of the distribution and pathogenicity of these protozoa. This paper treats the distribution of parasites found in sampling miscellaneous birds of the District of Columbia and vicinity and contains a preliminary report on the more important phase of the problem of pathogenicity.

METHODS

The work herein discussed was begun in the summer of 1938 incidental to a study of the cellular elements of avian blood and the data presented do not cover the entire bird population of the area. Had the bird traps been set near our laboratory in the city instead of in the suburbs, the catch would doubtless have resulted in fewer grackles and more starlings, which would probably have changed the total parasite count, since the numbers of parasites found in these two species differ so markedly. The game birds were bred on game farms in the vicinity and the ducks were taken from refuges not far away. Most of the smaller birds were trapped and released as soon as they were bled from the wing vein. A few of the birds were sick and sent in for diagnosis, and a few died in captivity.

Diagnosis was made from peripheral blood smears from live birds, except in a few cases where death of the bird made tissue smears available. The dried blood films and tissue smears were stained with Wright's stain, and examined for at least 20 minutes under oil immersion lens before they were declared negative for parasites.

Hybrid species of birds were not noted. This may account for the large number of purple grackles, some of which may have been crossed with bronze. The latter species occurs in this locality late in summer. Doubtful species of grackles were classed with the purple. More than 75 per cent of the birds were taken in the summer months.

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TABLE 1.—*Distribution of parasites in hosts*

HOSTS				PARASITES								
						Plasmodium						
Classification and name	Common name	No. examined	No. infected	*Haemoproteus*	*Leucocytozoon*	Round	Long	Mixed	Undetermined	*Toxoplasma*	Microfilaria	*Trypanosoma*
ANSERIFORMES												
ANATIDAE												
Cygnus columbianus	Whistling swan	3	1	1	0	0	0	0	0	0	0	0
Branta c. canadensis	Canada goose	13	1	1	0	0	0	0	0	0	0	0
Anas p. platyrhynchos	Common mallard	59	21	19	1	0	0	0	0	0	2	0
Mareca americana	Baldpate	1	1	0	1	0	0	0	0	0	1	0
Dafila acuta tzitzihoa	American pintail	8	4	0	4	0	0	0	0	0	2	0
Nyroca valisineria	Canvas-back	1	1	0	0	0	0	0	0	0	1	0
Nyroca affinis	Lesser scaup duck	3	2	0	0	0	0	0	0	0	2	0
FALCONIFORMES												
CATHARTIDAE												
Cathartes aura septentrionalis	Turkey buzzard	79	18	14	2	0	0	0	0	0	9	0
ACCIPITRIIDAE												
Buteo p. platypterus	Broad-winged hawk	1	1	1	0	0	0	0	0	0	0	0
Buteo b. borealis	Eastern red-tailed hawk	5	2	2	1	0	0	0	0	0	0	0
GALLIFORMES												
PERDICIDAE												
Colinus v. virginianus	Eastern bob-white quail	93	2	1	0	0	1	0	0	0	0	0
MELEAGRIDIDAE												
Meleagris gallopavo	Domestic turkey	4	1	1	0	0	0	0	0	0	0	0
COLUMBIFORMES												
COLUMBIDAE												
Zenaidura macroura carolinensis	Mourning dove	5	4	3	2	0	1	0	0	0	0	0
STRIGIFORMES												
STRIGIDAE												
Otus asio naevius	Eastern screech owl	7	5	1	3	0	2	0	0	0	1	0
Bubo v. virginianus	Great horned owl	1	1	1	0	1	0	0	0	0	0	0
Strix v. varia	Northern barred owl	3	2	1	2	1	1	0	0	0	0	1
PICIFORMES												
PICIDAE												
Centurus carolinus	Red-bellied woodpecker	1	1	0	0	0	1	0	0	0	0	0

TABLE 1.—(*Continued*)

HOSTS				PARASITES								
						Plasmodium						
Classification and name	Common name	No. examined	No. infected	*Haemoproteus*	*Leucocytozoon*	Round	Long	Mixed	Undetermined	*Toxoplasma*	Microfilaria	*Trypanosoma*
PASSERIFORMES												
HIRUNDINIDAE												
Progne s. subis	Purple martin	2	1	0	1	0	0	0	0	0	0	0
CORVIDAE												
Cyanocitta c. cristata	Northern blue jay	29	10	4	5	0	0	0	1	0	0	0
Corvus b. brachyrhynchos	Eastern crow	16	7	2	1	1	1	0	0	0	4	0
MIMIDAE												
Dumetella carolinensis	Cat bird	7	3	2	0	0	1	0	0	1	1	0
Toxostoma rufum	Brown thrasher	12	7	5	0	1	1	1	0	0	0	0
TURIDIDAE												
Turdus m. migratorius	Eastern robin	7	5	0	1	0	4	0	1	0	1	0
Hylocichla mustelina	Wood thrush	4	1	0	1	0	0	0	0	0	0	0
Hylocichla guttata faxoni	Eastern hermit thrush	2	1	1	0	0	0	0	0	0	1	0
Sialia s. sialis	Eastern bluebird	3	2	0	1	0	2	0	0	0	1	1
STURNIDAE												
Sturnus v. vulgaris	Starling	32	2	0	0	0	0	0	2	0	0	0
PLOCEIDAE												
Passer d. domesticus	English sparrow	9	1	0	0	0	0	0	0	1	0	0
ICTERIDAE												
Agelaius p. phoeniceus	Eastern red-wing	5	2	1	0	0	0	0	1	0	0	0
Quiscalus q. quiscula	Purple grackle	89	55	21	41	5	1	3	5	7	5	2
Quiscalus quiscula aeneus	Bronzed grackle	1	1	1	1	0	0	0	0	0	0	1
Molothrus a. ater	Eastern cowbird	5	1	0	0	0	0	0	0	0	1	0
THRAUPIDAE												
Piranga erythromelas	Scarlet tanager	1	0	0	0	0	1	0	0	0	0	0
FRINGILLIDAE												
Richmondena c. cardinalis	Eastern cardinal	8	5	1	4	0	0	0	3	0	0	0
Pipilo e. erythrophthalmus	Red-eyed towhee	6	1	0	0	0	0	0	1	0	0	0
Junco h. hyemalis	Slate-colored junco	29	3	0	2	0	1	0	0	0	0	0
Spizella p. passerina	Eastern chipping sparrow	2	1	0	1	0	0	0	0	0	0	0
Zonotrichia albicollis	White-throated sparrow	19	10	6	3	0	3	0	0	0	0	0
Passerella i. iliaca	Eastern fox sparrow	9	1	0	1	0	0	0	1	0	0	0
	Totals	585	188	90	79	9	21	4	15	9	32	5

RESULTS

A total of 618 birds, comprising 23 families and 53 species, was examined. Of these, 188 were parasitized with from one to five types of parasites, a total of 268 infections in all birds. The parasites identified were *Leucocytozoon, Haemoproteus, Plasmodium, Toxoplasma, Trypanosoma,* and microfilaria. Table 1 shows the distribution of these parasites based on a study of the morphology in the blood films.

DISCUSSION

No attempt was made to assign the parasites to species except in a few cases where sufficient gametocytes were found to permit average measurements and comparison with described species. For the most part, the infections were very light. The heaviest appear to have been in the brown thrashers and in a barred owl. The latter bird carried five distinct species of blood protozoa. One blue bird brought in sick from Beltsville had four species of blood parasites. Nine grackles, including one in juvenile plumage, showed triple infections, and the remaining birds had double or single infections. Of 20 young grackles, 12 were infected, nine of the 12 were infected with leucocytozoa.

One each of the following species was examined but found negative for blood parasites: Cooper hawk, red-shouldered hawk, downy woodpecker, red-headed woodpecker, yellow-breasted chat, song sparrow and meadow lark. Ten ringnecked pheasants were also negative as were five black vultures, three mocking birds, two sparrow hawks, two chimney swifts, two olive-sided fly catchers and two tufted titmice.

Plasmodium

Of the 53 *Plasmodium* infections herein reported, 15 were of undetermined type. In this group were placed those that showed only a few trophozoites which might have belonged to one of several species. It is not impossible that a few of them may have been *Haemoproteus* infections. Chronic cases of avian malaria, like human malaria, are sometimes impossible to detect by peripheral blood smears, especially in those species in which the parasite level is extremely low after the crisis is past, as in *P. cathemerium* and *P. circumflexum.* If a bird carries an infection for life, as is believed, chronic cases would be met with more often than acute ones in a general survey. To determine more accurately the extent of avian malaria in nature, blood inoculations should be made from the questionable bird into canaries, or better into clean birds of the same species as the host. Since this method was used in only five cases in this survey, these figures for *Plasmodium* infections represent only an estimate of the probable infection in this number of birds. One *P. elongatum* infection in a screech owl and one grackle *Plasmodium* were transmitted to canaries.

Of the 15 undetermined species of *Plasmodium*, five were in the grackles. Two of these appeared to be of similar type and different from any described species. An attempt to transmit one of these by blood and tissue inoculations resulted in an atypical infection, which is still under study in our laboratory. A total of 21 test birds—10 quail, 4 mallards, 4 pigeons, and 3 grackles—was used before a positive result could be obtained. These negative results may be due less to host specificity of the parasite than to the relative natural immunity to any *Plasmodium* of the species of birds selected for the experiment. Manwell and Herman (1935), however, also reported unsatisfactory results in estimating the *Plasmodium* infections in grackles, using a canary and an English sparrow for recipients of infected blood.

Plasmodium elongatum in the Bobwhite Quail (*Colinus virginianus virginianus*)

One quail not more than six months old was found to have a *Plasmodium* infection shortly after it was brought to the laboratory from nearby Maryland. Nine others brought in at the same time from the same place showed no parasites. There was no protozoan infection in the laboratory prior to this. Furthermore, it was in winter when mosquitoes are not active. The infection was discovered in December and continued for one month, after which no parasites could be found in the peripheral blood. Transmission tests to two canaries one year later, using 0.2 cc citrated blood intramuscularly gave negative results. Since birds are known to recover from this species of *Plasmodium*, this was considered to be a case of natural recovery.

The gametocytes of this quail *Plasmodium* differ slightly from the original description of *P. elongatum* Huff. Wolfson (1939) has demonstrated the gametocyte variation in a strain of *P. relictum* in the unnatural host, the duck, which variation has also been observed by the writer in a grouse *Plasmodium* when studied in quail and canaries. The inner margins of the gametocytes of this quail *Plasmodium* are often irregular, the ends sometimes appear to be square instead of round and they do not extend from one apex of the cell to the other. They do tend to bend around the host cell nucleus at one pole only, and sometimes increase the size of the host cell along the longitudinal axis. Trophozoites occupying a polar position are frequently found in immature red blood cells. These forms and adult gametocytes were fairly numerous in the blood slides, but segmenting forms were not found. These characteristics are the criteria for designating this parasite, *Plasmodium elongatum* Huff. Attempts to infect three quail with *P. elongatum* by blood inoculations from two screech owls proved unsuccessful. The injection of sporozoites from these owl infections might have given positive results, however,

since the quail herein reported must have received its infection in this manner. Dr. E. Allen (1931) reported finding *Plasmodium* in a group of quail from Georgia. "*P. praecox* is believed to be the causal organism of malaria in these birds since the gametocyte is elongate and partially surrounds the nucleus of the red cell." Dr. Allen, in a recent conversation with the writer, is of the opinion she also had a *P. elongatum* infection since she was doubtless using the nomenclature of Hartman (1927).

Haemoproteus

The *Haemoproteus* may be divided as were the *Plasmodium,* on the basis of the shape of the gametocyte, into three groups: (a) those that surround the host cell nucleus and fill the host cell cytoplasm, i.e., the *H. danielewsky* type; (b) those that grow on one side of the cell and displace the host cell nucleus, i.e., the *H. columbae* type; and (c) those that grow on one side of the host cell nucleus causing little or no hypertrophy of the cell and no displacement of the nucleus, i.e., *H. lophortyx*. Obviously this is an unsatisfactory method of classification since there is much variation between parasites of different ages, even in the same species. Mixed infections may be missed.

Of the 90 *Haemoproteus* infections found in this study, six were of the *H. danielewsky* type, while the remainder was about evenly divided between *H. columbae* type, and *H. lophortyx* type. Twenty-one of the 22 cases in grackles were of the latter type, while 16 of the 19 infections in mallards were of the *H. columbae* type, with two *H. danielewsky*, and one *H. lophortyx* type.

Three of the brown thrashers had sufficiently heavy infections to enable satisfactory measurement of the parasites and to identify them as belonging to *H. beckeri* species. Two mourning doves infected with *H. sacharovi* also had a few gametocytes that completely surround the host cell nucleus as well as a round type leucocytozoon infection which will be discussed later.

Haemoproteus of the Mallard (*Anas platyrhynchos platyrhynchos*)

Haemoproteus sp. has been described from the black duck by Herman (1938), who stated that it was the first report of this genus from a North American duck. The mallards from the vicinity of Washington are found to have three types of *Haemoproteus,* the most frequent of which is the *H. columbae* type. The parasites are usually numerous in the blood, and the incidence of infection varies from five out of nine parasitized in one sampling, to one in 12 infected the following summer.

The measurements of these parasites are based on the average figures from five mallards, 25 microgametes and 25 macrogametes being counted. The gametocytes are usually in contact with the host cell periphery, but

often do not touch the host cell nucleus, though it may be displaced laterally. The granules are large, black to brown, scattered in the macrogamete and polar in the microgamete.

The macrogametes stain deep blue with Wright's stain, and measure 12.6 to 16.1 microns in length and 2.6 to 4.9 microns in width, and average 14.1 by 3.7 microns. The nucleus stains pink, is usually round but sometimes triangular in shape, and is located on the periphery of the parasite nearest the host cell periphery. It varies in size from 1.4 to 4.1 microns at the widest part, and averages 2.8 microns. The granules number 8 to 26, the average being 17.6. The microgametes take very little stain, appearing hyaline to very light blue. They are 12.6 to 15.4 microns in length, and 2.8 to 5.6 microns in width, and they average 14.3 by 3.7 microns. The nucleus, when distinguishable, is a pale pink rectangle, centrally located. It averages 3.5 by 8.4 microns in size. The granules vary from 6 to 32 in number, and average 15.7 for the microgamete.

This *Haemoproteus* differs from that described from the black duck in being shorter and not usually wide enough to fill the host cell on one side. It also has a larger number of granules. It is similar in displacing the host cell nucleus.

Haemoproteus from the Turkey Vulture (*Cathartes aura septentrionalis*)

S. T. Darling, working in the Canal Zone, in 1912, reported finding *H. danielewsky* in a turkey buzzard *Cathartes aura.* He described it briefly but did not measure the parasite. He stated that the pigment of the microgamete had a tendency to be collected discretely in each pole. This does not read like a description of the true *H. danielewsky,* in which the gametocytes surround the host cell nucleus and fill the host cell. The description was not accompanied by illustrations. The parasite found in the turkey vulture taken near Washington, D. C., differs somewhat from Darling's description and is believed to be the first record of *Haemoproteus* from turkey vultures in the United States.

The parasites grow on one side of the host cell in close contact with the host cell periphery, not touching the host cell nucleus, but sometimes displacing it very slightly. They are frequently vacuolated. The macrogametes measure 13.3 to 15.7 microns in length and 3.1 to 4.2 microns in width; they average 14.3 by 3.8 microns. The nucleus is usually oval, centrally located, and may be either near the outer margin of the host cell or near the host cell nucleus. It averages 3 microns in size at widest part and stains pink. The macrogametes stain deep blue. The granules, varying in size from fine dots to large bacelloid structures, are scattered throughout the cytoplasm and vary in number from 8 to 38, with an

average of 27. The microgametes stain very lightly. The average measurements are 14.4 by 4.2 microns, varying from 13.3 to 16.1 microns in length and 3.3 to 4.4 microns in width. The nucleus is rarely apparent. The granules average 21 in number and are also scattered.

Haemoproteus from the Bobwhite Quail (*Colinus virginianus virginianus*)

Haemoproteus was found in the tissues of one bobwhite quail after death. Because the gametocytes of this genus of protozoa are known to round up preparatory to fertilization as soon as the blood cools, little can be said regarding morphology of this species. A number of forms which seemed little distorted were found to bear some resemblance to *H. lophortyx* of the valley quail. The host cell nucleus was displaced slightly but not to the extent of that of *H. columbae*. The parasite was usually in close contact with the host cell periphery, but seldom in contact with the host nucleus. One end was often broader than the other, the nucleus was oval, centrally located, and the scattered granules varied in number from 7 to 20. This is believed to be the first record of the genus *Haemoproteus* in bobwhite quail.

Haemoproteus of the Purple Grackle (*Quiscalus quiscula quiscula*)

Twenty purple grackles were found to harbor very light infections of the *H. lophortyx* type. Coatney and West (1938) have described and illustrated a *Haemoproteus* from the bronzed grackle from the vicinity of Peru, Nebraska, which they named *H. quiscalus* and classed as the *H. columbae* type. This parasite in the purple grackle displaces the host cell nucleus very little, in some cases not at all; it is somewhat smaller than *H. quiscalus* and exhibits little tendency to curve about the nucleus of the host cell. Since in the District of Columbia and vicinity bronzed and purple grackles, as well as hybrids, are found in the same flocks late in summer, it would not be surprising to find them carrying the same species of parasites. Five of these infections were found in birds in juvenile plumage early in the summer before the bronzed grackle had arrived. The gametocytes varied in length from 11.2 to 15.4 microns, and in width from 2.0 to 3.8 microns, and usually filled the host cell on one side. The nucleus of the macrogamete is characteristically located near one end of the parasite near the host cell periphery. The microgamete nucleus is centrally placed and diffuse. Sometimes the central vacuole described by Coatney and West for the macrogamete could be found. The granules were large, bacelloid, and varied from 6 to 12 in number. Although these linear dimensions are short compared with those given for *H. quiscalus* of the bronzed grackle, the close relationship of the hosts taxonomically being considered, these parasites in the

purple grackle are sufficiently like those described by Coatney and West to call them *H. quiscalus* Coatney and West.

Leucocytozoon

The basis for classification of the *Leucocytozoon* is the change in the morphology wrought by the parasite on the host cell, rather than the morphology of the parasite itself. Generally birds of the large orders are parasitized by the type of *Leucocytozoon* which produces fusiform or spindle shaped host cells, while the smaller birds are infected with the round host cell type. Mixed infections are encountered in the larger orders. Of the 79 *Leucocytozoon* infections reported herein seven of them are of the fusiform type. Three of the seven infections are mixed with round forms also. One young screech owl, one barred owl, and one pintail were so infected. The long forms in the screech owl were somewhat different from those in the duck and barred owl in having a bar of host nuclear material on either side of the parasite. The *Leucocytozoon* in the purple martin was different from the other 61 round forms in passerine birds in being more oval in form and causing less distortion of the host cell, neither spindle forms nor round forms being present. Böing (1925) illustrates similar forms in the partridge (Rebhuhn) (Figs. 11, 12 and 13, Plate I) and considers them to be developmental stages in the fusiform type. Giovannola (1936) shows a similar form in a microphotograph (Fig. 12, Plate XII) from a green linnet (*Coccothraustes vulgaris*) and considers it a new species. This infection in the purple martin was very light and though search was made for some indication that it might belong to the fusiform type none was found.

Leucocytozoon of the Purple Grackle (*Quiscalus quiscula quiscula*)

Forty-two of 90 grackles examined were infected with leucocytozoa, which produced more or less round gametocytes in a round host cell. The host nuclei varied in shape from a small triangular, dark-purple body, which looked like a wedge in the parasite, to a band that extended almost around the entire circumference of the parasite. In rare cases the host cytoplasm clung in patches around the nucleus and stained much paler than normal. The macrogametes stained bright blue and varied in size from round bodies about 9.8 microns in diameter to irregular ovals 12.6 by 11.2 microns. The smaller forms were more nearly spherical than the larger (possibly older) forms. The oval nucleus stained pink in varying degrees of intensity, sometimes appearing to have no stain at all. It was located at any place in the parasite, usually from the central to outer margin of the parasite away from the host cell nucleus. In less than one-fourth of the parasites a bright-red-staining karyosome could be demonstrated on the periphery of the nucleus, sometimes just outside,

sometimes just inside, its border. In some instances also there was a single vacuole near the border of the nucleus, as well as numerous vacuoles over the parasite. The microgametes averaged 12 by 8.8 microns and stained very light blue. The pale pink nucleus was often so indistinct as to make measurements impossible. In some cases it almost filled the parasite. The size was approximately 5.6 to 12 microns. In some microgametes a series of vacuoles was observed on the edge of the parasite inside the host nucleus cap. These vacuoles varied in number from 2 to 8 and were of various sizes.

Leucocytozoon of the Barred Owl (*Strix varia varia*)

As is shown in Table 1, this was a case of a double infection with leucocytozoa, both round and fusiform host cell types being present in small numbers. The fusiform type in the barred owl is similar to the figures of *L. ziemanni* of the little owl *Athena noctua* after Reichenow in Wenyon's Protozoology. The host cells drawn out to gently tapering ends average 40.6 microns in length, varying from 33.6 to 46.2 microns. The host cell nucleus was a thin red band curving about half of the oval parasite and varied in length from 22.4 to 25.2 microns. The microgametes average 20.3 microns in length and 5.6 microns in width, and had a large indistinct, pale central nucleus that filled a large part of the body of the light-blue parasite. The macrogamete stained deep blue, was somewhat wider than the microgamete, and had a smaller nucleus. It averaged 22.2 microns in length and 8.4 microns in width. No karyosome could be seen.

Leucocytozoon of the Mourning Dove (*Zenaidura macroura carolinensis*)

Two mourning doves were found with double infections of round type *Leucocytozoon* and *Haemoproteus sacharovi*. Novy and MacNeal (1905) describe the mature gametocytes of the latter as spherical, and suggest that this species of *Haemoproteus* was first observed by Sacharoff who regarded it as a *Leucocytozoon*. The round gametocytes of these mourning doves were found to lack pigment granules, and the host cell nucleus curved about the parasite, points which differentiate this from the genus *Haemoproteus*. Furthermore, one of these doves was kept under observation for one month during which time blood slides were made at intervals. The *Haemoproteus* disappeared for days at a time while gametocytes of *Leucocytozoon* could be found in varying stages of growth at almost every examination. The *Leucocytozoon* in the mourning doves appear to be the same as the species described for the purple grackle and other birds of the Order PASSERIFORMES. This is believed to be the first record of *Leucocytozoon* from North American COLUMBIDAE.

Leucocytozoon of the Turkey Vulture (*Cathartes aura septentrionalis*)

Leucocytozoon of the round host type were found in two turkey buzzards. In one bird the parasites were very scarce and showed pressure from the surrounding cells, a characteristic not observed in the passerine birds. However, the size appeared to be greater than the leucocytozoa of the smaller birds. Macrogametes from the second vulture so infected measured 16.8 μ in length and 11.2 μ in width, somewhat larger than similar species in the purple grackle. The cytoplasm of these parasites had the same granular structure, a karyosome could sometimes be demonstrated, and the host cell nucleus formed a cap on one side of the parasite or extended half way around it.

Toxoplasma

For some years workers have been observing avian species of *Toxoplasma,* rarely in the peripheral blood, more often in the internal organs, recognizable by characteristic morphology in the monocytes and lymphocytes as a single oval, blue-staining body, with a red granular nucleus, lying within a notch in the host cell nucleus. In the internal organs, where reproduction is said to take place by binary fission, a mass of dividing forms resembles schizonts of plasmodium. Because these parasites are so similar to some forms of the recently recognized exoerythrocytic stages of some of the avian malarias, they are being intensively studied at present by a number of workers, and it is hoped that their taxonomic position may be clarified thereby.

The nine cases reported in this survey were diagnosed from peripheral blood smears alone, and no dividing forms were found. *Toxoplasma* found in the purple grackles were similar to Marullaz's (1913) Fig. 1, p. 325, of *Toxoplasma avium,* to Fig. 13, plate 3, of Manwell's (1939) *Toxoplasma,* in a canary and appear to be the same as Type II *Toxoplasma* of Wolfson (1940). The parasites were rare in the blood smears, and in view of the fact that they may have some relation to other protozoa, it is of interest to note their occurrence with other parasites. Of the nine cases herein reported, all except one in the English sparrow were associated with some other type of blood parasite. A *Toxoplasma* infection occurred in a catbird that was also infected with *Haemoproteus.* Of seven grackle infections with *Toxoplasma,* two were associated with *Leucocytozoon* only, one with *Haemoproteus* only, three with *Leucocytozoon* and *Plasmodium* and one with *Leucocytozoon* and *Haemoproteus.*

Trypanosoma

Species of avian trypanosomes have been named on morphological grounds, although they are known to be pleomorphic, and it is not known

how many species of birds a single type of trypanosome may infect. These parasites are usually difficult to demonstrate in the peripheral blood smears, but bone marrow smears and culture methods will yield a higher incidence of infection. Packchanian (1939) states that in his culture studies of wild birds the percentage of trypanosomiasis is 80 to 82. Five infections report herein from 615 birds examined demonstrate the inferiority of dried blood films for diagnosing this infection in birds.

The trypanosomes found in two purple grackles and a bronze grackle appeared to be the same kind as *T. avium,* having myonemes, a rather wide undulating membrane, and short flagella. The average length of four forms without the flagellum was 35 microns and the width 7.3 microns. The oval pink-staining nucleus averaged 4.2 by 5.6 microns. The trypanosomes of the barred owl and the blue bird were too poorly stained to justify description. They appeared to have three or four undulations in the membrane and were without myonemes.

Microfilaria

These parasites were found in the blood stream of approximately five per cent of the birds examined. Little is known of the pathogenicity of the avian types. They have been observed in large numbers at autopsy, but what their relation to the cause of death may be is not known. Because some are periodic in appearance in the blood stream, the presence of a large number in a bird may be regarded as a periodic "shower" of these larvae. Thirty-two microfilarial infections were observed in 14 species of birds. All five species of ducks were hosts for this parasite. In five cases a careful search for the adult worm was made but none was found. *Diplotriaena* sp. are common filariae of crows, thrushes, and grackles, according to Dr. E. E. Wehr of the U. S. Bureau of Animal Industry. Filaria have also been described from *"Turdus* sp.," from ducks, from blue birds and cow birds. Further search may reveal nematodes in all these species of birds which show microfilaria.

A few characteristics of some of the forms found are recorded in Table 2. A single mallard harbored what is believed to be a double infection. The size range of the two types overlapped somewhat, but the longer forms were wavy, even curled, and were blunt at both extremities. The shorter forms were straight, pointed at the posterior, and had a large vacuole near the posterior end. The latter type, also, has been observed more commonly in mallards from other localities. A single robin found dead on the Refuge carried two sizes of microfilaria. The shorter forms were very rare in the lung and heart smears; the longer forms fairly numerous.

Purple grackles were found to harbor two types of microfilaria, the

TABLE 2.—*Microfilaria, measurements and characteristics*

Host	No. of forms measured	Length *microns*	Width *microns*	Anterior	Posterior	Sheath
Catbird	3	67.2 to 91 Av. 77.9	2.8 to 5.6 Av. 4.6	Blunt	Tapering	None
Crow	6	150 to 207.2 Av. 176.8	3 to 5.6 Av. 4.4	Blunt	Blunt to tapering	None
Grackle	3	133 to 154 Av. 146	4.2 to 5.6 Av. 4.9	Blunt	Blunt	None
Grackle No. 493	4	122.5 to 173.3 Av. 144	4.2 to 7 Av. 5.4	Blunt	Pointed	Some had visible cross striations
Hermit thrush	4	126 to 182 Av. 154	4.2 to 6.3 Av. 5.2	Blunt	Tapering	None
Mallard	8	116.2 to 175 Av. 150.1	4.2 to 5.6 Av. 4.9	Blunt	Blunt	None. Wavy
Mallard	8	89.6 to 109.2 Av. 98.2	4.2 to 5.6 Av. 5.2	Blunt	Pointed	None. Straight, large vacuole near pointed end
Robin	6	497 to 599 Av. 520	5.6 to 9.8 Av. 7.9	Blunt	Tapering	None. Wavy. Some had cross striations
Robin	2	154 and 168	7.0	Blunt	Tapering	None. Straight
Screech owl	7	154 to 245 Av. 201.9	2.8 to 4.2 Av. 3.7	Tapering	Tapering	None. Some cross striations
Vulture	6	51 to 82.6 Av. 62.3	2.8 to 4.2 Av. 3.5	Blunt	Pointed	None. Some appeared to have spicule on posterior end.

more common one being unsheathed and blunt at both ends. One grackle had a few posterior pointed forms as well as some with visible cross striations.

SUMMARY

Blood parasites were found in 30 per cent of the 618 birds examined. A total of 268 infections, representing six genera of parasites, was found.

Haemoproteus, most commonly observed, was found in approximately 15 per cent of the birds examined. The following new hosts are named for this genus: Broad-winged hawk (*Buteo p. platypterus*), red-tailed hawk (*Buteo borealis borealis*), bobwhite quail (*Colinus v. virginianus*), whistling swan (*Cygnus columbianus*), Canada goose (*Branta canadensis canadensis*), and hermit thrush (*Hylocichla guttata faxoni*).

Leucocytozoon occurred in approximately 13 per cent of the birds examined. The following new hosts are recorded for the round type: Turkey vulture (*Cathartes aura septentrionalis*), barred owl (*Strix v. varia*), purple grackle (*Quiscalus q. quiscula*), fox sparrow (*Passerella i. iliaca*), cardinal (*Richmondena c. cardinalis*), mourning dove (*Zenaidura macroura carolinensis*), and purple martin (*Progne s. subis*).

Plasmodia were found in 8 per cent of the birds examined. These new hosts are reported: Red-bellied woodpecker (*Centurus carolinus*), fox sparrow (*Passerella i. iliaca*), and scarlet tanager (*Piranga erythromelas*).

Toxoplasma were found in one per cent of the birds examined, and one new host—purple grackle (*Quiscalus q. quiscula*)—was recorded.

Trypanosoma were found in 0.8 per cent of the birds. The purple grackle (*Quiscalus q. quiscula*) and the barred owl (*Strix v. varia*) are named as new hosts for these parasites.

Microfilaria were found in 5 per cent of the birds. New hosts are: Turkey vulture (*Cathartes aura septentrionalis*), screech owl (*Otus asio naevius*), catbird (*Dumetella carolinensis*), hermit thrush (*Hylocichla guttata faxoni*), and robin (*Turdus m. migratorius*).

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NEW GENERA AND SPECIES OF THE FAMILY MONORCHIIDAE (TREMATODA), WITH A DISCUSSION OF THE EXCRETORY SYSTEM

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MONORCHIIDAE were frequently encountered during a study of the TREMATODA of marine fishes of Beaufort, N. C., in the summer of 1939. Most of the species found were obviously different from any previously named, but similar to unnamed species described and figured by Linton (1905). Although it was originally planned to describe only the excretory systems of the known species of Beaufort trematodes, it seems necessary first to name four new species and two new genera of monorchiids. All specimens were first studied alive in diluted sea water and then killed in heated Gilson's fluid; later the preserved specimens were mounted in gum damar. Drawings were made of all species, both from living specimens and from whole mounts. The monorchiids studied all belong to three genera; one of these, *Genolopa,* is an old genus and the other two are described for the first time in this paper.

Diplomonorchis n. g.

Monorchiidae with undivided pouch-like excretory bladder; two testes, symmetrically or obliquely located on sides of body; ovary trilobate, on right side of median line, anterior to level of testes or between them; vitellaria at level of testes with some follicles extending anterior and posterior to testes; uterus coiling between testes and along sides of body anterior and posterior to testes; genital pore median ventral, between crural fork and ventral sucker; cirrus and distal part of metraterm armed with spines. Flame cell formula $2[(2+2)+(2+2)]$. Parasites in intestine of sea fishes.

Type species, *Diplomonorchis leiostomi* n. sp.

Diplomonorchis leiostomi n. sp.

(Figs. 1, 2, 3a)

With characters of the genus. Length 0.40 to 0.80 mm, average about 0.60 mm. Width 0.25 to 0.45 mm, average about 0.35 mm. Oral sucker spherical, 0.06 to 0.09 mm in diameter, average dimensions about 0.08 by 0.08 mm. Ventral sucker in middle third of body, much smaller than oral sucker, 0.04 to 0.06 mm by 0.04 to 0.06 mm. (Average 0.05 by 0.045.) Pharynx nearly spherical, about 0.04 by 0.04 mm. Esophagus usually shorter than pharynx. Intestinal crura reaching far behind testes, nearly to posterior end of body. Excretory bladder pouch-shaped, tapering to a very narrow anterior end reaching to level of ovary. Collecting tubes joining anterior end of bladder on each side, dividing at level of ventral sucker; flame cell formula $2[(2+2)+(2+2)]$. Testes two, symmetrical or somewhat oblique, on

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sides of body lateral to intestinal crura; level of testes about half-way between ventral sucker and posterior end. Ovary ventral and median to right intestinal cecum, partly anterior to level of testes and partly between them, divided into three distinct lobes. Vitellaria at level of testes and extending anterior and posterior to testes, dorsal to intestinal crura, consisting of a group of eight to ten follicles on each side. Uterus coiling along sides of body anterior and posterior to testes, and also filling most of intercrural space behind level of ventral sucker. Eggs thick-shelled, yellow-brown, 28 to 30 μ long and 17 to 20 μ wide in living specimens and 22 to 27 μ by 14 to 18 μ in whole mounts (See Fig. 3a). Genital pore median, just behind level of crural fork. Cirrus pouch one-fourth to one-third length of body, enclosing short nearly spherical seminal vesicle, narrow tubular pars prostatica, numerous large prostate glands, and eversible cirrus, about one-third length of cirrus pouch, armed with many short spines shaped like rose thorns about 6 μ long and 6 μ wide. Metraterm pouch nearly as long as cirrus pouch, containing a sac-like proximal part and a distal part armed with spines which are like the cirrus spines in size and shape (See Figs. 9b, 9f).

Host: Spot, *Leiostomus xanthurus* Lacépède, and also occasionally in pigfish, *Orthopristis chrysopterus* (Linnaeus).

Location: Small intestine.

Locality: Beaufort, N. C.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 36777.

This species is represented by 136 specimens collected during the summer of 1939; it was found in 10 of the 19 spot and in 2 of the 6 pigfish examined. *Diplomonorchis* differs from *Monorcheides* (Odhner, 1905) principally in the shape of the excretory bladder (undivided instead of **Y**-shaped) and also in the position of the vitellaria (in the posterior half of the body instead of anterior to the ventral sucker). The only other genera of the MONORCHIIDAE with two testes are *Physochoerus* Poche, 1925, and *Paramonorcheides* Yamaguti, 1938, which like *Monorcheides* have vitellaria anterior to the ventral sucker. Manter (1940) describes the new species *Paramonorcheides bivitellosus,* remarking that "the long ceca and the posterior group of vitelline follicles of the present species might almost be of generic value." Manter's species must be transferred to *Diplomonorchis;* its name therefore becomes *Diplomonorchis bivitellosus* (Manter, 1940). *D. leiostomi* differs from *D. bivitellosus* by the smaller size of the ventral sucker (its diameter being little more than half that of the oral sucker, while in Manter's species the suckers are nearly equal), the more rounded forms of the testes, and the much larger eggs (22 to 27 μ long, compared with 19 to 20 μ in *D. bivitellosus*).

Postmonorchis n. g.

Monorchiidae with pouch-like undivided excretory bladder; loops of uterus mostly lateral to intestinal crura, not extending posterior to testis; testis single, in posterior part of body; vitellaria between levels of ventral sucker and testis; intestinal crura reaching nearly to posterior end of body; ovary on right side of median line; Laurer's canal and small seminal receptacle present. Cirrus and distal portion of metraterm armed with spines; uterus opening into the spiny distal part of metraterm pouch. Flame cell formula 2[(2+2) + (2+2)]. Intestinal parasites of sea fishes. Type species, *Postmonorchis orthopristis* n. sp.

Postmonorchis orthopristis n. sp.

(Figs. 7, 8, 3b)

Length of unflattened whole mounts 0.35 to 0.75 mm, width 0.30 to 0.50 mm, average dimensions about 0.62 by 0.38 mm. Cuticula covered with very minute spines. Oral sucker almost spherical, much larger than ventral sucker; oral sucker about 0.10 mm in diameter, slightly wider than long; ventral sucker about 0.05 to 0.06 mm, usually nearly circular. Prepharynx shorter than pharynx. Pharynx elliptical, 0.045 to 0.050 by 0.040 to 0.045 mm. Esophagus usually shorter than pharynx. Intestinal crura reaching to posterior edge of testis, or nearly to posterior tip of body. Excretory bladder an undivided pouch reaching to level of transverse vitelline ducts. Collecting tubes entering anterior end of bladder on each side; each collecting tube dividing at level of ventral sucker into an anterior and a posterior branch, each of which, by branching again, gives off two secondary branches with 2 flame cells on each branch; flame cell formula $2[(2+2)+(2+2)]$. Testis single, usually in posterior third of body, median, wider than long, posterior margin with median notch. Ovary smaller than testis, margins entire or indented, on right side of median line just in front of testis. Laurer's canal and small seminal receptacle present. Vitellaria consisting of two compact groups of about 8 follicles, on each side, close to dorsal surface, just anterior to level of testis; transverse vitelline ducts joining to form vitelline reservoir in median line at level of ovary. Uterus with very compact coils almost entirely in space between intestinal crura and lateral margins of body, never entering intercrural zone behind testis; eggs with thick yellow-brown shell, 18 to 20 μ by 13 to 15 μ in live specimens and about 17 by 12 μ in whole mounts (See Fig. 3b). Genital pore median, close in front of ventral sucker. Cirrus pouch about one-third as long as body, enclosing seminal vesicle, very narrow pars prostatica, strongly developed prostate glands, and a short eversible cirrus armed with numerous narrow wedge-shaped spines (Fig. 9a) 10 μ long and about 3 μ wide. Metraterm pouch nearly as long as cirrus, containing a distal part lined with numerous slender wedge-shaped spines and a sac-like proximal part making up about three-fifths of its length. Uterus opening into spiny part of metraterm pouch either laterally or medially.

Host: Pigfish, *Orthopristis chrysopterus* (L.); also spot, *Leiostomus xanthurus* Lacépède.

Location: Intestine.

Locality: Beaufort, N. C.

Syntype specimens: U. S. Nat. Mus. Helm. Coll. No. 36778.

This species is represented by 104 specimens from 5 of the 6 pigfish examined, and 2 specimens from 2 of the 19 spot examined. *Postmonorchis* differs from *Genolopa, Paraprototrema, Prototrematoides, Bivesicula, Bivesiculoides, Telolecithus,* and *Asymphylodora* in having most of the uterine folds outside of the intestinal ceca and none behind the testis; it differs from *Monorcheides, Physochoerus, Paramonorcheides,* and *Diplomonorchis* in having only a single testis; it differs from *Asymphylodora* in having the genital pore median instead of lateral, and it differs from *Monorchis* in having an undivided excretory bladder and vitellaria posterior to the ventral sucker. The "*Monostomum* sp." of Linton (1905), described on p. 379 and shown in his Fig. 222, is apparently *Postmonorchis orthopristis.*

There are now 13 genera in the family MONORCHIIDAE: *Monorchis* Looss, 1902, *Monorcheides* Odhner, 1905, *Asymphylodora* Looss, 1899, *Genolopa* Linton, 1910 (syn. *Proctotrema* Odhner, 1911), *Physochoerus*

Poche, 1925, *Telolecithus* Lloyd and Guberlet, 1932, *Bivesicula* Yamaguti, 1934, *Paraproctotrema* Yamaguti, 1934, *Proctotrematoides* Yamaguti, 1938, *Paramonorcheides* Yamaguti, 1938, *Bivesiculoides* Yamaguti, 1938, *Diplomonorchis* Hopkins, 1941, and *Postmonorchis* Hopkins, 1941. Two other genera, *Lasiotocus* Looss (in Odhner, 1911) and *Pristisomum* Looss (in Odhner, 1911), have been named but have never been described. The following key includes all the genera which have been described.

KEY TO THE GENERA OF MONORCHIIDAE

1 (4) Ventral sucker and blind metraterm pouch lacking; excretory bladder V-shaped 2
2 (3) Uterus extending posterior to testis .....*Bivesicula*
3 (2) Uterus not extending posterior to testis .....*Bivesiculoides*
4 (1) Ventral sucker and blind metraterm pouch present; excretory bladder undivided or Y-shaped 5
5 (6) Genital pore on margin of body, lateral to ventral sucker ..*Asymphylodora*
6 (5) Genital pore median or nearly median, anterior to ventral sucker 7
7 (14) Two testes present 8
8 (13) Vitellaria in anterior half of body, lateral or anterior to ventral sucker, not extending posterior to testes 9
9 (12) Testes near middle of body, uterus filling body behind testes 10
10 (11) Testes nearly spherical .....*Physochoerus*
11 (10) Testes longitudinally elongated, length more than twice width. *Paramonorcheides*
12 (9) Testes in posterior third of body, uterus not extending behind testes. *Monorcheides*
13 (8) Vitellaria mostly in posterior half of body, close to testes, with some follicles extending posterior to testes .....*Diplomonorchis*
14 (7) Only one testis present 15
15 (16) Vitellaria anterior to ventral sucker, excretory bladder Y-shaped with short stem and long furci .....*Monorchis*
16 (15) Vitellaria not extending anterior to ventral sucker, excretory bladder an undivided pouch 17
17 (18) Vitellaria behind level of testis .....*Telolecithus*
18 (17) Vitellaria anterior or lateral to testis 19
19 (20) Testis in posterior end of body, uterus not looping posterior to testis. *Postmonorchis*
20 (19) Testis some distance from posterior end, uterus filling body behind testis 21
21 (22) Muscular bulb surrounding terminal part of uterus where it enters metraterm .....*Paraproctotrema*
22 (21) No muscular bulb surrounding distal part of uterus 23
23 (24) Vitellaria in longitudinally elongated, almost linear arrangement, ovary smooth-margined; a muscular spiny pouch opening into common genital atrium, separate from metraterm pouch .....*Proctotrematoides*
24 (23) Vitellaria in compact, more or less rounded groups; ovary somewhat lobate in unflattened specimens; no muscular spiny atrial pouch separate from metraterm pouch .....*Genolopa*

Two of the genera included in this key, *Bivesicula* Yamaguti, 1934 and *Bivesiculoides* Yamaguti, 1938, probably should not be placed in the family MONORCHIIDAE; they are monostomes with distinctly V-shaped excretory bladders, and completely lack both the spiny cirrus and the blind metraterm pouch characteristic of all other genera which have been placed in this family.

The question of the probable synonymy of *Proctotrema* Odhner, 1911 with *Genolopa* Linton, 1910 has been discussed by several authors, but no definite conclusion has been reached. Lloyd and Guberlet (1932) discuss this question at length, concluding that "without more detailed information concerning the morphology of the several species of *Genolopa,* especially as to the type of excretory system and the presence or absence of a seminal receptacle, any revision of the genus is, of course, impossible." Yamaguti (1934) remarks, in discussing *Proctotrema:* "This genus is probably identical with *Genolopa* . . . because it seems almost certain that Linton overlooked the true acetabulum lying behind the genital pore. If, however, Linton is right on this point, then Odhner's genus should remain valid." Manter (1940, p. 407) comments: "It is probable that the genus *Genolopa* should be restricted to monorchids with a median cluster of much longer spines in the cirrus sac, a character of *G. ampullacea,* the type species. Such a view, however, would remove most of the species now contained in the genus." In a footnote on p. 402 of the same publication, Manter says: "The removal of *Genolopa truncata* from the genus *Genolopa* leaves *Genolopa ampullacea,* the type species, still in the genus which is at least for the present recognized as distinct from *Proctotrema.*"

I have examined Linton's type specimen of *Genolopa ampullacea* (U.S.N.M. Coll. No. 8525). This is a flattened specimen, well stained and quite adequate for study of all features that can be seen in a whole mount (See Fig. 4). There are two other specimens on the same slide; the larger of these is undoubtedly *G. ampullacea,* but the smaller specimen differs in several features and may possibly belong to a distinct species. The excretory bladder cannot be distinguished on any of these specimens. The type specimen agrees with Odhner's (1911) description of the genus *Proctotrema* in every point except for the more rounded shape of the oral sucker, the non-lobate form of the ovary (which may be a result of flattening, since the ovary in other species with tri-lobate ovary is more rounded in flattened specimens), and the less elongate shape of the eggs in *G. ampullacea.* Since these are minor differences, and since other species completely cover the range of differences in even these characteristics, it is obvious that *Proctotrema* Odhner, 1911 is a synonym of *Genolopa* Linton, 1910. In spite of Manter's statement, the spination of the cirrus in *G. ampullacea* is in no way exceptional, and there is no "median cluster of much longer spines in the cirrus sac" in the type specimen. *Proctotrema bacilliovatum* therefore becomes *Genolopa bacilliovatum* (Odhner, 1911), *P. lintoni* becomes *Genolopa lintoni* (Manter, 1931), *P. plectorhynchi* becomes *Genolopa plectorhynchi* (Yamaguti, 1934), *P. macrorchis* becomes *Genolopa macrorchis* (Yamaguti, 1934), *P. longicaecum* becomes *Genolopa longicaecum* (Manter, 1940), and

P. costaricae becomes *Genolopa costaricae* (Manter, 1940). The other species of *Genolopa* are *G. truncata* Linton, 1910, *G. trifolifer* Nicoll, 1915, *G. cacuminata* Nicoll, 1915, *G. minuta* Manter, 1931, *G. elongata* Manter, 1931, and two new species described in the present paper, a total of 14 species; *Genolopa ampullacea* Linton, 1910 is the type species.

The species of *Genolopa* studied at Beaufort in the summer of 1939 are described below.

Genolopa lintoni (Manter, 1931)

Forty-one specimens, from 5 of the 6 pigfish (*Orthopristis chrysopterus*) examined, were referred to this species. They varied from a length of 1.0 mm to 1.7 mm. The ventral sucker in my specimens is slightly more than half the diameter of the oral sucker, the pharynx is 0.05 to 0.07 mm long and 0.05 to 0.06 mm wide, the esophagus is nearly as long or longer than the pharynx, and the ceca extend to the posterior half of the testis or slightly beyond. Other characteristic features are shown in Figs. 10, 11, 3e and 9e, h. No seminal receptacle was seen, but it may nevertheless be present, as the arrangement of organs would make it very difficult to see this structure if present. The eggs average about 23 by 13 μ. The most striking feature of this species is the comparatively large size of the rose-thorn shaped cirrus spines (25 μ long) and the long spike-shaped spines of the metraterm (25–30 μ long); the spines of the external body covering, however, are of the same size as those in *Genolopa beauforti* (10 μ long).

Manter was evidently mistaken in saying that the "Monostomum sp." of Linton, 1905, p. 379, is a synonym of *G. lintoni*. Apparently the species referred to is the one shown in Linton's Fig. 223, for Manter quotes Linton's statement that the eggs are three times longer than wide. I have found numerous specimens of the species shown in Linton's Fig. 223, and they are very different from *G. lintoni*, as will be seen from the following description.

Genolopa longovatum n. sp.

(See Figs. 12, 3c, 9c)

Length usually between 0.5 and 1.0 mm but sometimes slightly exceeding these limits. Body urn-shaped, broadest in middle, tapering toward both ends but expanding again at the anterior end around the large oral sucker. Cuticular spines covering entire body. Oral sucker slightly more than twice diameter of ventral sucker (oral sucker in type 0.12 by 0.15 mm, ventral sucker 0.05 by 0.06 mm). Pharynx nearly spherical, about two-thirds diameter of ventral sucker. Prepharynx very short, seldom visible in whole mounts. Esophagus slightly longer than pharynx. Intestinal ceca ending about half-way between testis and posterior end. Single testis very close behind ventral sucker, sometimes partly dorsal to it, so that cirrus pouch is crowded forward and seldom extends posterior to ventral sucker. Cirrus and distal portion of metraterm pouch spiny; spines of cirrus wedge-shaped, nearly in form of equilateral triangle, about 8 μ long; spines of metraterm very narrow and needle-like (Fig. 9c). Ovary on right side of ventral sucker, overlapping anterior end of testis, slightly trilobate to almost spherical. Vitellaria consisting of compact groups of 8 to 10 follicles close to dorsal surface on each side of ventral sucker and anterior end of testis. Coils of uterus filling intercecal space from ovary to posterior end, slightly overlapping intestinal ceca and edges of testis. Eggs elongate, averaging 26 by 11 μ, and in living specimens often 2½ times as long as wide (Fig. 3c). Excretory bladder undivided, pouch-shaped.

Host: Pigfish (*Orthopristis chrysopterus*).
Location: Intestine.
Locality: Beaufort, N. C.
Type specimen: U. S. Nat. Mus. Helm. Coll. No. 36780.

The most distinctive features of this species in life are the urn-like shape, the very elongate eggs, and the almost funnel-like form of the oral sucker. The flame cells and excretory tubules were seen only once; the flame cell formula appeared to be $2[(2+2)+(2+2)]$, but this cannot be stated as a fact until more observations can be made. *G. longovatum* is distinguished from all other species except *G. bacilliovatum* (Odhner, 1911) and *G. costaricae* (Manter, 1940) by the elongate form of the eggs, which are however not as long as those of Odhner's species. *G. longovatum* differs from *G. bacilliovatum* in having a much greater difference in the relative sizes of the two suckers and in having a less distinctly lobed ovary, as well as in having shorter eggs (26 μ as compared with 31–33 μ) and shorter cirrus spines (8 μ compared with 35 μ). *G. longovatum* has longer ceca than *G. costaricae,* and differs also in body shape. This is almost certainly the same species as the specimen shown in Fig. 223 of Linton (1910) under the name of "*Monostomum* sp." *G. longovatum* occurred in the intestines of all of the six pigfish (*Orthopristis chrysopterus*) examined at Beaufort; a total of 54 specimens was collected.

Genolopa beauforti n. sp.

(See Figs. 5, 6, 3d, 9d, g)

Body elongate, length four to seven times width, covered with cuticular spines about 10 μ long. Length of mature specimens 1.2 mm to 2 mm, width 0.25 to 0.35 mm. Oral sucker shaped like inverted bell with upper half twice as wide as lower half, 0.15 to 0.20 mm long and 0.13 to 0.16 mm wide; ventral sucker spherical, 0.08 to 0.11 mm in diameter, diameter about half length of oral sucker. Prepharynx longer than esophagus, nearly as long to twice length of pharynx. Pharynx very large, usually larger than ventral sucker. Esophagus short to twice as long as pharynx. Intestinal ceca reaching to posterior end of body. Genital pore at anterior edge of ventral sucker, slightly lateral to median line. A very long common genital sinus, sometimes longer than metraterm pouch, without spines. Cirrus pouch extending behind ventral sucker about half way to testis; cirrus armed with triangular spines about 8 μ long, distal portion of metraterm pouch armed with slender spines about 18 μ long. Uterus opening into metraterm pouch at junction of armed and unarmed portions, which is anterior to middle of pouch; loops of uterus filling all space between other organs from metraterm pouch to posterior end, but only slightly overlapping the intestinal crura. Ovary on right side just anterior to testis, nearly spherical with a slight tendency toward triangular shape. Testis about half way between ventral sucker and posterior end. Vitellaria consisting of compact groups of 8 to 10 follicles, on each side of ovary. Eggs 18 to 20 μ long and 12 to 15 μ wide. Excretory bladder a short undivided pouch reaching less than half way to testis; main collecting vessels dividing at level of ventral sucker into an anterior and a posterior branch; flame cell formula $2[(2+2)+(2+2)]$.

Host: Pigfish (*Orthopristis chrysopterus*).
Location: Intestine.
Locality: Beaufort, N. C.
Type specimen: U. S. Nat. Mus. Helm. Coll. No. 36779.

Twelve specimens of *G. beauforti* were found, in 3 of the 6 pigfish examined. This species is easily distinguished by its elongate form, long prepharynx, very large pharynx, peculiarly shaped oral sucker, and long intestinal ceca reaching to posterior end of body. *G. beauforti* is more elongate than any other species except *G. elongata* (Manter, 1931) and *G. cacuminata* Nicoll, 1915 both of which have ventral suckers larger than oral suckers. The pharynx of *G. beauforti* is larger (in relation to the sizes of the suckers) than in any other species.

THE EXCRETORY SYSTEM OF MONORCHIIDAE

The excretory systems of four species, in four different genera, are now completely known. These are *Diplomonorchis leiostomi* Hopkins, 1941, *Postmonorchis orthopristis* Hopkins, 1941, *Genolopa beauforti* Hopkins, 1941, and *Monorcheides cumingiae* (Martin, 1938). The life cycle of the last species is the only one known in this family. Martin (1939) found that *Cercaria cumingiae* Martin, 1938, a cercaria with eyespots but without stylet and with a fairly long undivided tail bearing peculiar lateral lappets instead of spines or setae, encysts in the clam which serves as first intermediate host, and becomes adult in the anterior intestine of eels and flounders. It is interesting to note that the excretory bladder of *Monorcheides cumingiae* (at least in the cercaria and metacercaria stages) has an undivided bladder like that of *Genolopa*, a fact which casts doubt on Odhner's statement that *Monorcheides* has a Y-shaped bladder. All of the known excretory systems have the flame cell formula $2[(2-2)-(2-2)]$, which we may assume with some confidence to be the formula for all members of the family MONORCHIIDAE.

Members of several other families have the flame cell formula $2[(2-2)-(2-2)]$. In the MICROPHALLIDAE, species of the genera *Microphallus, Cornucopula, Spelotrema, Levinseniella,* and *Maritrema* have been shown by various authors to have this formula. In the HETEROPHYIDAE, *Caecincola,* according to Lundahl (1939) and *Centrocestus,* according to Yamaguti (1938a) have the $2[(2-2)-(2-2)]$ formula. MacFarlane (1939) reports this formula for *Coitocaecum,* a member of the COITOCAECIDAE or OPECOELIIDAE. Dobrovolny (1939) found the same formula in *Plagioporus* of the family ALLOCREADIIDAE, and I have found it in the allocreadiid-like genera *Helicometra* and *Cymbephallus* (unpublished observations). *Tergestia,* in the STERINGOPHORIDAE (= FELLODISTOMIDAE) also has the $2[(2-2)-(2-2)]$ formula (Hopkins, 1940). *Zoogonus,* in the ZOOGONIDAE, has been reported to have this formula by Stunkard (1938).

Considering the marked differences in their adult structures and life cycles, it seems unlikely that the possession of the same flame cell formula indicates close genetic relationships between all of these families. It

would be more plausible, perhaps, to suggest that the 2[(2 – 2) – (2 – 2)] formula represents a primitive condition usually passed over early in the embryonic development of cercariae, but that in many possibly unrelated genera the development of the excretory system has been retarded so that it remains in the primitive condition. On the other hand, if genera which had been placed in the same family were found to have widely different flame cell formulae, it would certainly cast doubt on the closeness of their relationship, especially if the difference were found in the cercaria as well as in the adult stages.

SUMMARY

1. The new genera *Diplomonorchis* and *Postmonorchis* are described.

2. The new species *Diplomonorchis leiostomi* (from *Leiostomus xanthurus*), *Postmonorchis orthopristis* (from *Orthopristis chrysopterus*), *Genolopa longovatum* (from *Orthopristis chrysopterus*), and *Genolopa beaufort*i (from *Orthopristis chrysopterus*) are described, all from Beaufort, N. C. Type specimens of all new species have been deposited in the U. S. National Museum.

3. *Proctotrema* Odhner, 1911 is declared a synonym of *Genolopa* Linton, 1910.

4. The excretory systems of *Diplomonorchis leiostomi*, *Postmonorchis orthopristis*, and *Genolopa beaufort*i are described, all with undivided excretory bladder and the flame cell formula 2[(2 – 2) – (2 – 2)].

5. Keys are presented for identification of the genera of the MONORCHIIDAE.

6. The possession of the 2[(2 – 2) – (2 – 2)] flame cell formula by genera in several other families is discussed.

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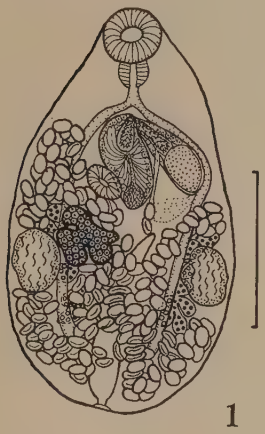

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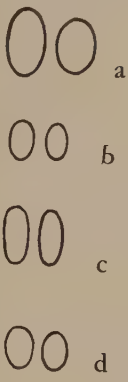

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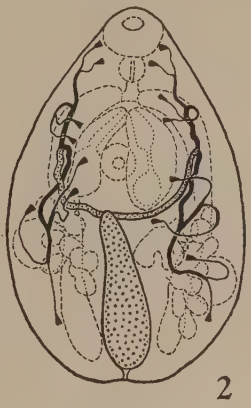

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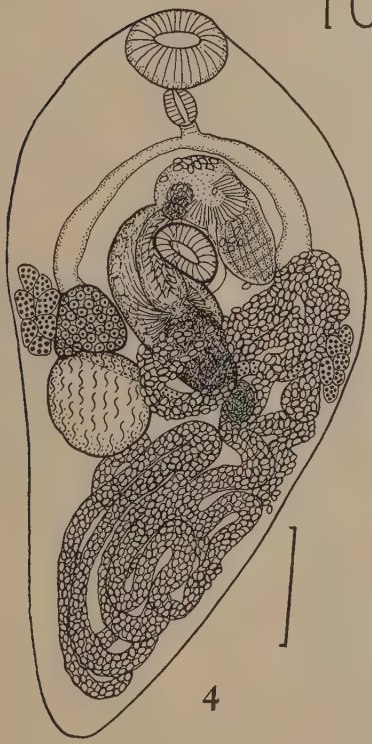

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PLATE I

Explanation of Plates

Plate I

Fig. 1. *Diplomonorchis leiostomi* n. sp., ventral view of whole mount. Scale line = 0.2 mm.

Fig. 2. *Diplomonorchis leiostomi,* camera lucida drawing of living specimen, dorsal view. Excretory tubules and flame cells drawn freehand.

Fig. 3. Camera lucida drawings of typical eggs of the 5 species described in this paper. The eggs on the left are drawn from living specimens, those on the right from whole mounts in gum damar. Scale line = 0.02 mm.

a, *Diplomonorchis leiostomi*
b, *Postmonorchis orthopristis*
c, *Genolopa longovatum*
d, *Genolopa beauforti*
e, *Genolopa lintoni*

Fig. 4. *Genolopa ampullacea* Linton 1910, type specimen, drawn with camera lucida, ventral view. Scale line = 0.2 mm.

Fig. 5. *Genolopa beauforti* n. sp., camera lucida drawing of whole mount, ventral view. Scale line = 0.2 mm.

Fig. 6. *Genolopa beauforti,* camera lucida drawing of living specimen, with excretory tubules and flame cells added freehand.

Plate II

Fig. 7. *Postmonorchis orthopristis* n. sp., camera lucida drawing of living specimen, with excretory tubules and flame cells added freehand.

Fig. 8. *Postmonorchis orthopristis,* camera lucida drawing of whole mount, ventral view. Scale line = 0.2 mm.

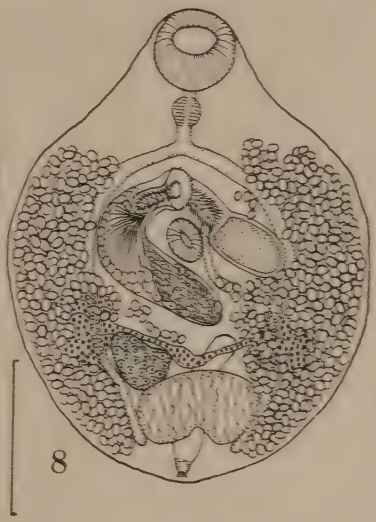
Fig. 9. Genital spines of the 5 species described in this paper; camera lucida drawings from living specimens. Scale line = 0.02 mm.

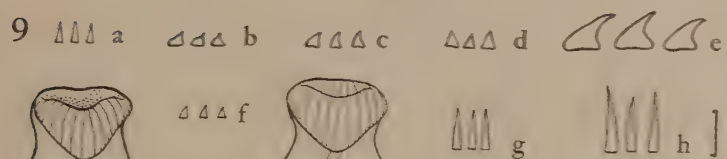
a, cirrus spines of *Postmonorchis orthopristis*
b, cirrus spines of *Diplomonorchis leiostomi*
c, cirrus spines of *Genolopa longovatum*
d, cirrus spines of *Genolopa beauforti*
e, cirrus spines of *Genolopa lintoni*
f, metraterm spines of *Diplomonorchis leiostomi*
g, metraterm spines of *Genolopa beauforti*
h, metraterm spines of *Genolopa lintoni*

Fig. 10. *Genolopa lintoni* (Manter, 1931), camera lucida drawing of whole mount, ventral view, with eggs omitted for the sake of clearness. Scale line = 0.2 mm.

Fig. 11. *Genolopa lintoni,* camera lucida drawing of whole mount, dorsal view. Scale line = 0.2 mm.

Fig. 12. *Genolopa longovatum* n. sp., camera lucida drawing of whole mount, ventral view. Scale line = 0.2 mm.





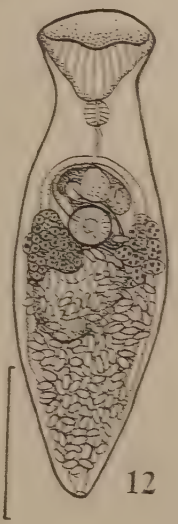


PLATE II

ON DISTRIBUTION RELATIONSHIPS BETWEEN CALIFORNIA TIDE POOL FISHES AND THEIR MYXOSPORIDIAN (PROTOZOAN) PARASITES

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Workers in the field of taxonomic parasitology are often impressed with the lack of correlation between the distribution of parasites and the distribution of their hosts. Some species of parasites are narrowly confined to a particular host species in one locality, while others have a distribution as wide as the earth's surface and involve many different species of hosts. The writer has become interested in variation in host-parasite relationships through investigations of myxosporidian parasites of tide pool fishes.

A description of a new species of myxosporidia will precede a discussion of distribution.

Sphaeromyxa lateralis n. sp.

(Figs. 1–3)

Trophozoite leaf-like, flattened. Length up to 1 and ½ millimeters. Periphery only slightly differentiated from endoplasm. Each pansporoblast develops two spores. Polysporous. Small ameboid stages occasionally present which have clear needle-like pseudopodia (Fig. 1), and measure 15 microns in total length. No movement observed.

Spore 26 microns long (suture diameter) by 8 microns wide; oval, slightly curved in side view. Polar capsules 6.3 by 8.6 microns. Polar filaments loosely coiled, approximately 22 microns long. The capsulogenous nucleus of one end usually takes a much darker stain (iron hematoxylin) than that of the other end of the spore. Sporoplasm lightly granular, with two large nuclei. Each nucleus with a single karyosome. Suture line indistinct. Figs. 2 and 3.

Host: Artedius lateralis Giard.

Location: Gall bladder.

Locality: Tide pools of Santa Barbara, California.

In comparison with the other species of the genus, *Sphaeromyxa lateralis* is most similar to *S. gibbonsia* Noble from the gall bladder of *Gibbonsia elegans elegans* and *G. metzi.* The principal difference is in the shape of the polar capsule which is markedly shorter and wider in *S. lateralis.* Only three infected hosts have been found. These were collected during November and December, 1939.

The gall bladders of *Dialarchus snyderi* Greeley from Santa Barbara (October 1937), Point Conception (November 1939 and August 1940) and from Santa Rosa Island (August 1940) were found to be heavily

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infected with *Myxidium incurvatum* Thélohan. This species has been reported from the Mediterranean Sea, the North Sea and from the American Atlantic and Pacific coasts. The characters of the parasite found in *Dialarchus snyderi* are as follows:

Trophozoite irregularly globular, with rounded, blunt pseudopodia (Fig. 4). Very slow ameboid movement. Pseudopodia slightly less granular than cytoplasm of body. Little distinction between ectoplasm and endoplasm. Latter with large refractive granules. Mature size 30 microns in diameter. Disporous.

Spore (Figs. 5 and 6) averages 4.5 by 11 microns; polar capsules average 1.8 by 3.8 microns. Spindle shape in front view, with very pointed ends; may be swelled in the middle. In side view the whole spore is twisted into an S-shape. The axes through the long diameters of the polar capsules are not parallel. Shell wall thin and smooth. Sporoplasm lightly granular and fills spore, with two nuclei which are indistinct in living spores. Suture line faint. Filaments not observed.

In his original description Thélohan (1892) described a disporous condition for *Myxidium incurvatum* from *Nerophis aequoreus*, *N. annulatus*, etc., from the southern coast of France, while Jameson (1929) described a monosporous condition for the same parasite in *Sebastodes caurinus* Richardson from Pacific Grove, California. The forms described above from *Dialarchus snyderi* are smaller than those described by Thélohan.

In the gall bladder of one specimen of *Gibbonsia elegans elegans* (Cooper) collected at Point Conception (October 1939) a heavy infection of *Leptotheca sphaerula* was found.

Gibbonsia metzi Hubbs was found to be the host of two other myxosporidia. In three of these fish from Point Conception (November 1939) the gall bladders were found to be infected with *Ceratomyxa blennius*. The only other reported infection of California tide pool fishes by this genus was by Jameson (1929). He described *C. gracilis* in *Gibbonsia metzi* (evides) from Pacific Grove. In the gall bladder of one specimen of *G. metzi* from Santa Barbara (April 1940) the writer found an abundance of *Trilospora californica* with a small number of *Leptotheca elegans*.

In the gall bladders of two specimens of the blind goby, *Typhlogobius californiensis*, from Santa Barbara (April 1940) were found moderate infections of *Leptotheca elegans*. These parasites were mixed with *Trilospora californica*.

Leptotheca elegans has also been found in the gall bladder of *Artedius lateralis* from Santa Barbara (December 1939) together with *Sphaeromyxa lateralis* described above. The former differed from the original description (Noble 1938) in that no movement of the trophozoite was observed, and the spores averaged 7 by 22 microns instead of 9 by 17 microns.

A list of tide pool fishes of California from which myxosporidia have

been described, together with their parasites, was published in a previous paper (Noble 1939). Herewith is the same list with the additions mentioned above.

HOST LIST WITH PARASITES

Arbaciosus rhessodon
  Sphaeromyxa ovula in gall bladder
Artedius lateralis
  Sphaeromyxa lateralis in gall bladder
  Leptotheca elegans in gall bladder
Auchenopterus intergripinnis
  Leptotheca elegans in gall bladder
Clinocottus analis
  Ceratomyxa gracilis in gall bladder
Dialarchus snyderi
  Myxidium incurvatum in gall bladder
Gibbonsia elegans
  Leptotheca elegans in gall bladder
  Sphaeromyxa gibbonsia in gall bladder
  Trilospora californica in gall bladder
  Leptotheca sphaerula in gall bladder
  Ceratomyxa blennius in gall bladder
Gibbonsia metzi
  Leptotheca sphaerula in urinary bladder
  Leptotheca elegans in gall bladder
  Sphaeromyxa gibbonsia in gall bladder
  Ceratomyxa gracilis in gall bladder
  Ceratomyxa blennius in gall bladder
  Trilospora californica in gall bladder
Hypsoblennius gilberti
  Ceratomyxa blennius in gall bladder
Rimicola eigenmanni
  Leptotheca compressa in urinary bladder
Typhologobius californiensis
  Trilospora californica in gall bladder
  Leptotheca elegans in gall bladder

The following list illustrates differences in infection range of myxosporidia among California tide pool fishes:

Ceratomyxa blennius
  Gibbonsia elegans
  Hypsoblennius gilberti
  Gibbonsia metzi
Ceratomyxa gracilis
  Gibbonsia metzi
Ceratomyxa obesa
  Clinocottus analis
Leptotheca compressa
  Rimicola eigenmanni
Leptotheca elegans
  Artedius lateralis
  Auchenopterus intergripinnis
  Gibbonsia elegans
  Gibbonsia metzi
  Typhologobius californiensis
Leptotheca sphaerula
  Gibbonsia elegans
  Gibbonsia metzi
Myxidium incurvatum
  Dialarchus snyderi
Sphaeromyxa gibbonsia
  Gibbonsia elegans
  Gibbonsia metzi
Sphaeromyxa lateralis
  Artedius lateralis
Sphaeromyxa ovula
  Arbaciosus rhessodon
Trilospora californica
  Gibbonsia elegans
  Gibbonsia metzi
  Typhologobius californiensis

Of particular interest, from the standpoint of distribution, is the presence of *Myxidium incurvatum* which has been found in only one species of fish in this locality, but which has been reported from at least sixteen different species of fish, including both fresh water and marine forms, from various parts of the world.

The littoral flora and fauna of the California Coast north of Point Conception is unlike the littoral flora and fauna south of Point Conception. This is due to a difference in temperature and direction of water and air currents in these two regions. Several species of fishes are common to both localities. From the point of view of host-parasite relationships it is of interest to know whether the distribution of the parasites of these fishes parallels that of their hosts.

For this study the gall bladders of tide pool fishes were examined, and their infection by myxosporidian parasites noted.

In comparing the myxosporidia of fishes occurring north of Point Conception with those occurring south of this Point, Jameson (1931) states that there is "marked similarity between the two areas." The writer has found, on the basis of the study of tide pool fishes alone, that a similarity is not very marked.

The gall bladders of approximately 125 specimens of *Clinocottus analis* from Point Conception southward have been examined by the writer, and no parasites have been found. These fishes were collected over a period of three years, and during all seasons of the year. Jameson (1929) described *Ceratomyxa obesa* from *Clinocottus analis* collected in Monterey Bay, approximately 175 miles north of Point Conception. The absence of parasites in the fishes from southern waters suggests at least a physiological difference between the two groups of hosts. The writer recently (August) collected 9 specimens of this fish from Monterey Bay, and was unable to find myxosporidia in any of them. There is evidence from the work of Bolin (unpublished) that a subspecific separation of the two groups of fish is unwarranted.

Dialarchus snyderi Greeley is abundant in tide pools along the entire coast of California. The writer has collected these fishes during all seasons of the year on both sides of Point Conception from Monterey Bay southward to Ventura. Infection of the gall bladders of *D. snyderi* with *Myxidium incurvatum* Thélohan is equally common in all localities studied. This condition is to be expected because of the world wide distribution of *M. incurvatum.*

Gibbonsia elegans (Cooper) and *Gibbonsia metzi* Hubbs are both abundant along the coast of California, and both harbor, with one exception, the same species of myxosporidian parasites. Here is an example of two distinct species of hosts, found under varying environmental conditions, which are both subject to infection by essentially the same gall

bladder parasites. From a study of the parasites of this genus the writer concludes that the hosts are physiologically more similar than is indicated by their structural characteristics.

Of the fishes extensively studied by the writer, *Montereya recalva* (Greeley) is the only common tide pool fish of central and southern California which is free from gall bladder parasites. Approximately 100 gall bladders were examined, taken throughout the year from Point Conception and the Channel Islands, and all were found to be negative for myxosporidia.

When we consider the bottom feeding habits of the tide pool fishes, with the abundance of algae and small crustaceans usually present, we find a combination of conditions particularly favorable to the development and transmission of the myxosporidia. Bangham and Hunter (1939) made a similar observation in their studies of fish parasites of Lake Erie. They found that the fish at the western end of the lake, which is shallower and weedier, supported greater numbers and greater variety of parasites when a fish was infected. Davis (1917) points out that "it is very common to find two or more species of myxosporidia living in the gall bladder or the urinary bladder at the same time."

Somewhat opposed to the above findings are those of Cross (1938) who concluded that "fish that harbor large numbers of parasites of one kind have a tendency toward a light infection, or none, with other parasites." A glance at the host list on page 411 shows that out of ten different species of tide pool fishes only two harbor more than two species of myxosporidian parasites. Even in the genus *Gibbonsia,* which harbors 6 different species of myxosporidian parasites, the writer usually found only one species of myxosporidia present in any one fish, and never more than two of these sporozoans, although occasionally other parasites (worms) were encountered. Thus the study of myxosporidia of tide pool fishes supports the conclusions of Cross.

The ability of a parasite to live normally in many different species of hosts indicates a pronounced adaptability on the part of both host and parasite. This adaptability could be explained on the basis of a generalized condition of the parasite. According to this interpretation the parasite has not progressed far enough, phylogenetically speaking, to attain the specialization which permits it to dwell in only one particular species of host. When we compare *Leptotheca elegans,* however, which is found in five different species of host, with *Leptotheca compressa,* which is confined to *Rimicola eigenmanni,* we can observe no differences which would indicate that the latter is more specialized. Since *L. elegans* has not been found in *Rimicola eigenmanni* we can assume that this fish and parasite are physiologically incompatible. If a difference in generalization of the above mentioned parasites exists it must be of a physiological nature.

The physiology of the myxosporidia has merited little attention on the part of investigators, and offers an open field for research.

SUMMARY

Sphaeromyxa lateralis n. sp. from the gall bladder of *Artedius lateralis* has been described. A revised list of tide pool fishes of California with their myxosporidian parasites, and a list of myxosporidian parasites of California with their hosts is included.

Investigations on the host-parasite problem with relation to distribution of tide pool fishes and their myxosporidian parasites result in the following conclusions:

1. Two groups of hosts, both belonging to the same species but separated each into a different environment, may sometimes be distinguished by differences in their parasitic fauna.

2. Some parasitic species are very resistant to change, and are able to maintain their diagnostic characteristics in several species of hosts in different environments.

3. There is often no close correlation between the structural similarities of two species of hosts and the similarities of the parasites with which they are infected.

4. Fishes living in the littoral zone, where there is an abundance of plant life, are generally infected with a greater variety of parasites than are pelagic fishes.

5. The presence of large numbers of parasites of one species in a fish usually excludes the presence of large numbers of other species of parasites in that fish.

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EXPLANATION OF PLATE

All figures drawn with the aid of a camera lucida. Magnification × 2230.

Fig. 1. Living trophozoite of *Sphaeromyxa lateralis.*

Fig. 2. Two living spores of *Sphaeromyxa lateralis.*

Fig. 3. Pair of stained spores of *Sphaeromyxa lateralis.* (Brazil's Bouin's, iron hematoxylin.)

Fig. 4. Young living trophozoite of *Myxidium incurvatum.*

Fig. 5. Two living spores of *Myxidium incurvatum.*

Fig. 6. Stained spore of *Myxidium incurvatum.* (Brazil's Bouin's, iron hematoxylin.)

COMPARATIVE EFFICIENCY OF ZINC SULFATE AND SUGAR SOLUTIONS FOR THE SIMULTANEOUS FLOTATION OF COCCIDIAL OÖCYSTS AND HELMINTH EGGS

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Zoological Division, U. S. Bureau of Animal Industry

A number of substances has been recommended for use in detecting protozoan cysts and helminth eggs in feces and soil. For many years, it has been known that certain of these substances were more efficient in floating protozoan cysts while others were more satisfactory in recovering helminth eggs. Until recently, however, little or no attempt was made to collect experimental data on the efficiency of substances for the simultaneous flotation of both protozoan oöcysts and helminth eggs from the same medium. Faust et al (1938, 1939) tested several techniques for the recovery of protozoan cysts and helminth eggs simultaneously from human feces and found that zinc sulfate solution having a specific gravity of 1.180 recovered the largest number of these forms. Garcia and Pesigan (1940) demonstrated that the IHP flotation method (named after Institute of Hygiene, Department of Parasitology, University of Philippines) involving the use of cupric nitrate having a specific gravity of 1.180 was just as effective in the diagnosis of helminth ova and protozoan cysts in human feces as zinc sulfate technique. These authors recommended the cupric nitrate technique for the following reasons: (1) smear preparations do not dry, even without the cover slip, for one month or more; (2) cupric nitrate preparations give a clearer and cooler visual microscopic field thus alleviating eye strain; (3) the fecal material is stained bluish-green while the eggs and cysts remain unstained; and (4) the high solubility of cupric nitrate gives a wider range of specific gravities than zinc sulfate. Pesigan (1940) tested both the zinc sulfate and cupric nitrate techniques for the simultaneous flotation of helminth eggs and protozoan cysts from human feces and found that each gave an efficacy of approximately 90 per cent.

The present paper presents data on the comparative efficiency of zinc sulfate and sugar solutions for the simultaneous flotation of coccidial oöcysts and helminth eggs from naturally infected and artificially inoculated chicken feces and soil.

MATERIALS AND METHODS

Tests were conducted on five fecal samples and five soil samples, the latter of a sandy loam nature. Three of the soil samples and two of the

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fecal samples were inoculated with known numbers of coccidial oöcysts and nematode eggs while the two remaining soil samples and the three remaining fecal samples, which were collected from infected poultry yards and infected poultry, respectively, contained unknown numbers of oöcysts and eggs.

Of the 3 inoculated soil samples, the first and second (Nos. 1 and 2 on chart) were collected from an area previously unused as a chicken yard; they were thoroughly dried in an oven at 50° to 60° C before being inoculated. An analysis[1] of a small portion of one of these dried samples showed that it contained 0.04 per cent nitrogen, 2.15 per cent organic matter, 16 per cent moisture, and had a pH of 4.2. A 125 cc portion of sample 1 was made into a paste by adding 25 cc of water; this reduced the volume of the dried soil to 74 cc. To this paste were added 193,750 oöcysts and 19,000 nematode eggs. A 109 cc portion of sample 2 was prepared in a manner similar to sample 1 except that 49,500 oöcysts and 22,400 nematode eggs were added.

The third inoculated soil sample (No. 3 in chart) was collected from the surface of the ground around the feeders and shelters of an infected poultry yard. An analysis of a portion of this latter sample, which had previously been comminuted and dried in an oven at a temperature of 50° to 60° C for more than a month, showed that it contained 0.27 per cent nitrogen, 4.16 per cent organic matter, 34 per cent moisture, and had a pH of 7.76. A preinoculation examination of several samples of this soil showed that an average of 14 oöcysts per 2 cc of soil were present. A 125 cc portion of this sample was mixed thoroughly with 63 cc of tap water; this increased the volume of the dried soil to 136 cc. To this paste there were added 121,000 oöcysts and 37,500 nematode eggs.

The fourth and fifth soil samples (Nos. 4 and 5) were scraped from the surface of the ground around the feeders and shelters of infected poultry yards. A small portion of each sample was comminuted and screened. The screenings from soil sample No. 4 were thoroughly mixed with water before being examined for eggs and oöcysts. Soil sample No. 5 was dried in an oven at a temperature of 50° to 60° C for a month and then inoculated with an unknown number of eggs and oöcysts before being tested.

Fecal samples (Nos. 6 and 7) were collected from parasite-free chickens. Ninety cc of sample No. 6 were inoculated with 413,200 oöcysts and 90,000 eggs. The volume of the inoculated sample was 96 cc. Sample No. 7 was dried in an oven at 50° to 60° C for one month, then removed and ground into a powder with a mortar and pestle. One hundred and twenty-five cc of the ground droppings were mixed into a smooth paste

[1] Analysis made by A. Yelmgren of the Division of Soil Chemistry and Physics, Bureau of Plant Industry.

with 150 cc of water; the volume of this mixture was 175 cc. To 135 cc of this paste there were added 121,000 oöcysts and 37,260 eggs, increasing the volume of the mixture to 170 cc.

Fecal samples (Nos. 8, 9, and 10) were collected from chickens naturally infected with coccidia and helminths. These chickens were confined in cages for a week and fed a mash free of grit before the samples were taken.

The prepared soil and fecal samples were placed in sealed jars which were kept in a refrigerator until ready for use.

Suspensions of sporulated oöcysts used to inoculate the fecal and soil samples were obtained by culturing the unsporulated oöcysts of *Eimeria maxima, E. mitis, E. acervulina*, and *E. tenella* in 2.5 per cent potassium dichromate solution for two to three days. On completion of sporulation, the oöcysts were washed free of potassium dichromate with tap water and then strained through cheesecloth and cotton to remove all debris. The number of oöcysts in each suspension was then determined from counts made with the aid of a Fuchs-Rosenthal blood counting chamber 0.2 mm deep. A pipette was used to stir the suspension of oöcysts to distribute them equally throughout the liquid before making each count. Counts on individual samples were continued until the numbers of oöcysts of two or more counts were identical. At least five and usually more counts were made of each suspension and then averaged. This average was then used to estimate the total number of oöcysts desired to be used in the inoculum.

The suspensions of helminth eggs were prepared by culturing the freshly collected eggs of the worms *Ascaridia lineata* and *Heterakis gallinae* in a 2 per cent solution of potassium dichromate for about two weeks. The egg suspension was then strained through cheesecloth to remove the debris and then washed free of potassium dichromate with tap water. Several counts were made of each suspension by the Stoll dilution method and the average of these used to determine the number of eggs desired to be used in the inoculum.

The inoculum was prepared by mixing thoroughly definite amounts of the egg and oöcyst suspensions. After inoculation, the soil or fecal sample was mixed thoroughly with a spatula to insure a uniform distribution of eggs and oöcysts.

The method adopted for the flotation of helminth eggs and protozoan cysts from fecal and soil samples was as follows: A 2 cc subsample, which was obtained by means of a U. S. Bureau of Plant Industry watch glass (27 mm in diameter and 8 mm high), was placed in a 125 cc Erlenmeyer flask containing 33 cc of tap water and glass beads. The contents of the flask were shaken vigorously for 5 minutes and then strained through 4 thicknesses of washed cheesecloth into two 15 cc centrifuge tubes. The

tubes were centrifuged at 1,050 RPM for 3 minutes. The supernatant liquid was poured off and a small amount of the flotation medium added to each tube. The contents of one tube were mixed thoroughly with an applicator stick and poured into the other. Enough of the flotation medium was then added to the latter tube to bring the level of the suspension even with the rim of the tube. A clean No. 2 coverslip was placed across the rim of the tube, care being taken to see that no air bubble or at most not more than a very small one was present underneath the slip. The tube was centrifuged at 1,050 RPM for 3 minutes. The coverslip was then removed to a slide for enumeration of eggs and oöcysts present thereon. This same procedure was carried out a second, sometimes a third or even a fourth time for the purpose of determining the total number of eggs and oöcysts recoverable from the subsample. Table I shows in tabulated form the results obtained from the examination of two coverslips of each sample tested as described above.

The zinc sulfate solution (sp gr 1.200), which was used in the present tests, was prepared by placing 331.4 grams of the chemical in a 1,000 cc glass cylinder and then adding enough water to bring the level of the solution to the one liter mark. The sugar solution (sp gr 1.270) was prepared according to the formula of Sheather (1923 a and b) and Benbrook (1925). Sugar solution having a specific gravity of 1.200 was made by adding 375 grams of sugar in 500 cc of water and 3.5 cc of phenol. A hydrometer was used to measure the specific gravity of all solutions at room temperature.

EXPERIMENTAL RESULTS

An examination of Table 1 shows that, in all tests in which a particular sample was inoculated with a known number of oöcysts and eggs, zinc sulfate consistently removed a larger number of oöcysts than the sugar solution (sp gr 1.270), while the latter, except in the case of soil sample No. 1, floated a few more helminth eggs. For every 100 oöcysts floated by the sugar solution (sp gr 1.270), 260 oöcysts were removed by the zinc sulfate solution, and for every 100 eggs floated by the zinc sulfate solution, 135 eggs were removed by the sugar solution. As regards sugar solution (sp gr 1.200), this medium proved to be slightly more efficient for the removal of eggs and slightly less efficient in recovering oöcysts than the zinc sulfate solution. For every 100 oöcysts floated by the sugar solution (sp gr 1.200), 114 oöcysts were floated by the zinc sulfate solution and for every 102 eggs floated by the zinc sulfate solution, 107 eggs were floated by the sugar solution (sp gr 1.200).

The foregoing results clearly demonstrate that each solution was more or less consistent in the relative number of eggs and oöcysts recovered from each of the samples, but was surprisingly inconsistent in the com-

TABLE 1.—*Flotation of eggs and oöcysts from soil (samples 1–5) and feces (samples 6–10)*

Sample No.	Av. inoculum per 2 cc sample		Zinc sulfate (sp. gr. 1.200)						Sugar (sp. gr. 1.270)						Sugar (sp. gr. 1.200)					
			Eggs			Oöcysts			Eggs			Oöcysts			Eggs			Oöcysts		
	Eggs	Oöcysts	No.[1]	Mean[2]	% Rec.[3]	No.	Mean	% Rec.	No.	Mean	% Rec.	No.	Mean	% Rec.	No.	Mean	% Rec.	No.	Mean	% Rec.
1	513	5,236	297 407 461	388	*75.7*	4,285 4,770 4,812	4,622	*88.3*	329 248 462 483	380	*74.2*	1,215 1,121 1,000 799	1,033	*19.7*						
2	411	908	83 100 91 123 113 143	108 (σ_m 9.1)[4]	*26.5*	108 122 126 145 132 147	130 (σ_m 6.0)	*14.3*							84 67 129 108 118 109	102 (σ_m 9.4)	*24.9*	90 108 142 96 84 111	105 (σ_m 8.4)	*11.6*
3	548	1,793	214 167 170	183	*33.5*	446 355 488	429	*23.9*	289 201 210	233	*42.6*	139 144 172	151	*8.5*						
4			100 93 110	101		387 302 490	393		220 202 261	227		326 208 396	310							
5			208 205 205 188 222 258	214 (σ_m 9.8)		645 792 623 560 477 647	624 (σ_m 42.8)		235 191 237 141 255 269	221 (σ_m 19.3)		177 336 370 129 491 320	300 (σ_m 55.0)		223 206 223 273 259 239	237 (σ_m 10.2)		674 467 499 479 753 759	605 (σ_m 56.7)	
6	1,875	8,608	243 139 281 281 294 256	249 (σ_m 24.0)	*13.2*	1,036 701 794 1,125 974 963	927 (σ_m 65.1)	*10.8*							387 263 344 299 287 241	303 (σ_m 23.2)	*16.2*	762 719 757 828 923 820	801 (σ_m 31.9)	*9.3*
7	438	1,423	72 49 95	72	*16.4*	110 172 140	140	*9.9*	107 83 149	113	*25.8*	61 102 81	81	*5.7*						
8			903 1,098	1,005		408 427	417		1,289 1,155	1,222		141 141	141							
9			0			4,144									1			4,036		
10			885			1,355									657			1,367		

[1] Each number represents the average number of oöcysts or eggs on first two coverslips.
[2] Mean counts for each sample.
[3] Percentage recovery.
[4] Actual differences in the means divided by the differences in the standard deviations of the means.

parative percentages of oöcysts and eggs recovered from the different types of samples. Zinc sulfate removed approximately 75 per cent of the eggs and 88 per cent of the oöcysts from soil sample No. 1 and only 26 per cent of the eggs and 14 per cent of the oöcysts from soil sample No. 2 which was of the same origin as sample No. 1; the only difference in the two samples was that sample No. 2 had been dried for a longer period and had been inoculated with a different suspension of eggs and oöcysts. In contrast, zinc sulfate recovered 33 per cent of the eggs and 24 per cent of the oöcysts from soil sample No. 3 which had been collected from a poultry yard and contained approximately 3 times as much organic matter as soil samples 1 and 2. Similar inconsistencies were found in the percentage recoveries of eggs and oöcysts from the different samples with sugar solutions. However, the oöcyst returns of the sugar solution (sp gr 1.200) more closely approximated those of the zinc sulfate. The percentage recoveries of eggs and oöcysts from fecal samples were consistently lower than those from soil samples. The highest percentage recovery of eggs from any fecal sample was 25.8 when sugar solution having a specific gravity of 1.270 was used and the highest percentage of oöcysts was 10.8 when zinc sulfate was used.

Only the results obtained from samples inoculated with known numbers of oöcysts and eggs were used in drawing conclusions as to the comparative efficiency of recovery of eggs and oöcysts by the techniques used. The tests with the samples containing unknown numbers of eggs and oöcysts demonstrated the practicability of the techniques in that they showed the same proportional yield as with the known samples.

DISCUSSION

In an attempt to explain the variation in percentages of recovery of eggs and oöcysts from different samples by the same technique, such factors as organic matter content, the size of the soil particles, and moisture content, have been considered. As stated earlier in the paper, an analysis showed that the samples differed in the amount of organic matter present. The percentage recoveries of eggs and oöcysts in soil samples 1 and 2 were markedly different, yet they were taken from the same source and possessed the same organic matter content. Moreover, the size of the particles in the samples apparently did not account for the difference in the percentage recoveries of eggs and oöcysts, since no better returns were obtained from the finely ground samples than from the coarsely ground material. Furthermore, the amount of moisture present in the mixed samples, which would influence the concentration of the subsamples, apparently had little to do with the percentage recovery of eggs or oöcysts from the subsamples. Soil samples 1 and 2 contained almost the same amount of moisture (about 17 per cent) but upon flotation these

samples yielded very different returns of eggs and oöcysts. A higher percentage of eggs and oöcysts was recovered from soil sample 3 than from 2, yet sample 2 contained less moisture than sample 3. In regard to the suspensions used for inoculation, the separate cultures of eggs and oöcysts no doubt varied in degree of sporulation or embryonation. However, the condition of the cultures does not seem to explain the fact that the yields of eggs and oöcysts increased or decreased proportionately for the different samples.

In this study it was also found that the number of oöcysts and eggs present on the first two coverslips represented a large majority of the recoverable forms. More than 70 per cent of the oöcysts recovered by the first two coverslips were present on the first coverslip when zinc sulfate solution was used while only slightly more than 50 per cent of oöcysts recovered by the first two coverslips were present on the first coverslip when sugar solution of either specific gravity was used. The reverse of this seemed to be true for the eggs. About 78 per cent of the eggs were present on the first coverslip in the case of zinc sulfate solution while over 90 per cent of the eggs were present on the first coverslip in the case of sugar solution (sp gr 1.200). There was recovered on the first coverslip approximately 82 per cent of the eggs when sugar solution (sp gr 1.270) was used.

Granting that zinc sulfate was somewhat more efficient for the recovery of oöcysts in the present test and that sugar solution of the same specific gravity (1.200) was slightly more efficient for the recovery of eggs from fecal and soil samples, the sugar solution is preferred to the zinc sulfate solution for simultaneous flotation of these organisms because: (1) it is more easily obtained; (2) it does not crystallize so rapidly, thus obviating the necessity of examining the slides at once or placing them in a moist chamber; and (3) the eggs and oöcysts are more evenly distributed over the slide.

SUMMARY

A study of results obtained by a modified D.C.F. (direct centrifugal flotation) examination of soil and fecal samples containing known and unknown numbers of eggs and oöcysts of chicken parasites warrants the following conclusions:

1. Zinc sulfate solution of a specific gravity of 1.200 is a more efficient medium for the centrifugal flotation of oöcysts of the chicken coccidia, *Eimeria acervulina, E. mitis, E. maxima,* and *E. tenella,* than a sugar solution of a specific gravity of 1.270. However, the zinc sulfate is less efficient for floating the eggs of *Ascaridia lineata* and *Heterakis gallinae.*

2. Sugar solution of a specific gravity of about 1.200 is probably a more efficient medium for floating eggs, but zinc sulfate of the same

specific gravity is probably more efficient for floating oöcysts in simultaneous centrifugal flotations of the eggs and oöcysts of the chicken parasites mentioned.

3. Although sugar solution (sp gr 1.200), is no more efficient than zinc sulfate solution (sp gr 1.200), it is more practical for the detection of the above poultry parasites because (1) it is more easily obtained than zinc sulfate, (2) it is less expensive; and (3) it is a better mounting medium.

4. The modified D.C.F. technique used herein, recovered from 10 to 88 per cent of the eggs and oöcysts inoculated into soil and fecal samples. There is need for further study of the factors which determine the efficiency of simultaneous flotation of helminth eggs and protozoan cysts in order to improve the technique of examining fecal samples from poultry and other domesticated animals.

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ORNITHODOROS PARKERI COOLEY: OBSERVATIONS ON THE BIOLOGY OF THIS TICK*

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Ornithodoros parkeri was first collected in Natrona County, Wyoming, by the writer in 1934 and was described by Cooley in 1936. Subsequent observations on its biology and distribution and its relationship to the transmission of relapsing fever spirochetes and certain other infectious agents are summarized below. The host and distribution data have been mostly obtained from materials collected by staff members of the Rocky Mountain Laboratory, but a few of the records are from collections by field crews of the Plague Laboratory at San Francisco, California.

DISTRIBUTION

Wyoming: Natrona, Carbon and Sweetwater Counties. Montana: Beaverhead, Madison and Ravalli Counties. Colorado: Moffat and Mesa Counties. Washington: Franklin, Yakima, Douglas and Okanogan Counties. Nevada: White Pine, Elko, Lyon, Lincoln, Clark and Churchill Counties. Utah: Uintah, Emery, Wayne, Washington, Grand, Carbon, and Iron Counties. California: Merced, Fresno and Kern Counties. Oregon: Benton and Umatilla Counties.

TYPE AREAS

Sagebrush prairies, grassy slopes, and semi-desert areas, from low elevations, as the San Joaquin Valley in California, to elevations of 7000 feet on plateaus in southern Wyoming.

HABITAT

Burrows and nests of rodents and burrowing owls.

NATURAL HOSTS

Prairie dogs (*Cynomys* sp.), jack rabbits (*Lepus* sp.), cottontail rabbits (*Sylvilagus* sp.), mice (*Peromyscus* sp.), ground squirrels (*Citellus* spp.), weasel (*Mustela* sp.), and the burrowing owl (*Speotyto cunicularia*). This tick has been most frequently found in association with prairie dogs.

EXPERIMENTAL HOSTS

These ticks feed readily on man, white mice, white rats, guinea pigs and monkeys.

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Four ticks, 3 males and one second stage nymph, have engorged on man experimentally; and one first stage nymph accidentally. There is no sensation while feeding. At the site of the bite there is a circumscribed hemorrhagic area with subsequent papule formation which may become necrotic. Intense itching occurs at intervals for 4 or 5 days.

FEEDING HABITS

Like *turicata* and *hermsi*, this species feeds rapidly. The male, when allowed to feed to repletion, becomes as fully distended as the female or any of the immature stages. The time required for engorgement depends on several factors, among which are: The interval which has elapsed since the last molt, the site of attachment, and the proximity of other attached ticks. Larvae, nymphs in all stages, and males and females may engorge in from 15 to 30 minutes. Feeding time determined for 20 males varied from 12 to 16 minutes.

COXAL FLUID

When engorgement is nearing completion, there is a copious amount of coxal fluid. It has not been shown that this fluid plays any essential rôle in the infective process in relapsing fever or spotted fever. Fluid from ticks shown to be infective for these diseases when injected into white rats or guinea pigs has not resulted in infection. In relapsing fever the host may become infected in less than one minute following attachment of the tick at a time when coxal fluid has not appeared.

LONGEVITY

The longevity of species of this genus is proverbial. Francis has recently reported the survival of *O. turicata* for 7 years. Late nymphs, males, and females of *parkeri* are still alive after approximately 4 years of fasting.

COPULATION TIME

The time was determined from the immobilization of the attached male to its voluntary detachment following the deposition of the spermatophore. Twenty-five such periods varied from 12 to 49 minutes. The average was 28 minutes.

MOLTING HABITS

Observations have been made on groups of *parkeri* from Montana, Wyoming, California, and Utah. When the larvae were engorged they were placed in individual cotton-stoppered shell glass vials, numbered serially, and kept at room temperature in a humidity jar containing a saturated solution of ammonium chloride. Higher temperatures hastened molting while low temperatures delayed it. Nymphs stored at a tempera-

ture of about 65° F failed to molt over a period of 3 months, but molted overnight when removed to a temperature of 97° F. Nymphs that are not completely engorged may molt regularly or may fail to molt until engorgement has taken place.

Observation on Montana stock, Table 1. September 11–13, 1937, 41 larvae engorged on a white rat. The limits for molting were 16 and 28 days, with the largest number molting by the 18th. Only 33 survived for the first nymphal feeding. The limits of nymphal molting were 14 and 41 days rather evenly distributed. In the second nymphal stage molting began on the 14th day and ended on the 31st, in the third it ranged from 15 to 32 days and in the fourth from 20 to 49 days. Twenty ticks survived to the adult stage. There were 16 males and 4 females. One of the males required only 3 nymphal stages to arrive to maturity while 15 males and the 4 females required 4 nymphal stages.

Observations on Wyoming stock, Table 2. July 28, 1938, 48 larvae engorged on a white rat. The molting period ranged from 8 to 12 days with 73 per cent molting on the eighth day. Only 35 ticks survived for the first nymphal feeding. The molting period varied from 13 to 18 days with the largest number molting on the 15th day. Thirty-four ticks survived for the second nymphal feeding. All but 2 molted in 14 to 19 days. These 2 molted on the 74th day. The third nymphal molts varied from 16 to 49 days, the fourth from 16 to 28 days, and one tick required a fifth molt which took place on the 25th day. Of the 34 ticks which survived to the adult stage 15 were males and 19 females. Three of the males and 2 females required only 3 nymphal stages, 12 males and 16 females, 4 nymphal stages, and one female 5 nymphal stages.

Observations on California stock, Table 3. December 4 to 6, 1939, 59 larvae engorged on white rats. All larvae molted in from 10 to 13 days with the largest number molting on the 11th day. Fifty-four ticks survived for the first nymphal feeding. The limits of the molting period were 11 and 19 days, with the largest number molting on the 14th. In the second nymphal stage the limits were 12 and 23 days, and in the third nymphal stage 13 and 20 days. Fifty-four ticks survived to the adult stage. Of these, 30 were males and 24 females. Eighteen of the males required only 2 nymphal stages, while 12 males and the 24 females required 3 nymphal stages. It is possible that the fewer nymphal stages required for maturity reflects the environmental conditions in central California.

Observations on Utah stock, Table 4. January 30 to February 20, 1940, 114 larvae engorged on white rats. The molting period varied from 7 to 13 days. Ninety-five ticks survived for the first nymphal feeding. The molting period ranged from 7 to 12 days. Ninety-three ticks survived for the second nymphal feeding. The molting period varied

TABLE 1.—Ornithodoros parkeri (*Montana stock*). *Molting data. Larvae engorged September 11 to 13, 1937*

Stage	Numerators: number of days after feeding required for molting. Denominators: number of ticks that molted in that number of days.	Total ticks
Larvae	16/1 17/6 18/15 19/4 20/8 21/5 22/1 28/1	41
First nymphal ..	14/2 15/1 16/6 17/4 18/2 19/4 21/2 23/1 35/1 38/3 39/5 40/1 41/1	33
Second nymphal	14/1 15/2 16/3 18/1 19/1 20/2 24/1 22/2 23/2 24/3 25/3 26/1 27/2 28/2 31/2	28
Third nymphal .	15/1 19/2 20/1 22/2 23/1 24/2 25/2(1♂) 26/1 29/3 30/1 32/3	19(1♂)
Fourth nymphal	20/2♂♂ 21/1♂ 23/2♂♂ 24/2♂♂ 26/2♂♂ 27/1♀ 28/3(2♂♂ 1♀) 29/1♂ 31/1♂ 35/1♀ 49/2(1♂ 1♀)	18 (11♂♂ 4♀♀)

TABLE 2.—Ornithodoros parkeri (*Wyoming stock*). *Molting data. Larvae engorged July 28, 1938*

Stage	Numerators: number of days after feeding required for molting. Denominators: number of ticks that molted in that number of days.	Total ticks
Larvae	8/35 9/10 10/1 11/1 12/1	48
First nymphal ..	13/3 14/8 15/10 16/7 17/5 18/2	35
Second nymphal	14/2 16/6 16/10 17/7 18/5 19/2 74/2	35
Third nymphal .	16/1 18/1 21/2 22/2 28/1 35/1 36/1 39/1 42/1♂ 43/1 45/5(1♂) 46/12(1♂ 1♀) 47/4(1♀) 49/1	34 (2♂♂, 2♀♀)
Fourth nymphal	16/3(2♂ 1♀) 17/1♀ 18/4(1♂ 3♀) 19/4(3♂ 1♀) 20/3(2♂ 1♀) 21/9(3♂ 6♀) 23/1♀ 25/2♀♀ 26/1♂ 28/1	29 (12♂♂, 16♀♀)
Fifth nymphal ..	25/1♀	1♀

TABLE 3.—Ornithodoros parkeri (*California stock*). *Molting data. Larvae engorged December 4 to 6, 1939*

Stage	Numerators: number of days after feeding required for molting. Denominators: number of ticks that molted in that number of days.	Total ticks
Larval	10/20 11/27 12/8 13/4	59
First nymphal .	11/1 12/2 13/10 14/21 15/7 16/7 17/2 18/3 19/1	54
Second nymphal	12/1 13/2 14/6 15/7(2♂♂) 16/10(2♂♂) 17/5(1♂) 18/11(3♂♂) 19/2♂♂ 20/5(4♂♂) 21/2♂♂ 22/1♂ 23/1♂	53 (18♂♂)
Third nymphal .	13/1♀ 14/3♂♂ 5♀♀ 15/4♂♂ 8♀♀ 16/1♂ 5♀♀ 17/4♂♂ 3♀♀ 19/1♀ 20/1♀	36 (12♂♂, 24♀♀)

TABLE 4.—Ornithodoros parkeri (*Utah stock*). *Molting data. Larvae engorged January 30 to February 20, 1940*

Stage	Numerators: number of days after feeding required for molting. Denominators: number of ticks that molted in that number of days.	Total ticks
Larval	7/6 8/32 9/50 10/19 11/5 12/1 13/1	114
First nymphal	7/10 8/56 9/23 10/3 11/2 12/1	95
Second nymphal	10/10 11/25 12/32 13/4 14/8 15/8 16/4 24/1 34/1	93
Third nymphal	15/1 16/3 17/12 18/6(1♂) 19/13(3♂♂) 20/13(6♂♂ 2♀♀) 21/15(7♂♂ 3♀♀) 22/8(5♂♂ 3♀♀) 23/8(3♂♂ 1♀) 24/3(2♂♂ 1♀) 25/3(1♂ 1♀) 26/2(2♂♂) 32/1 33/2 34/1 40/1 41/1	93 (30♂♂ 11♀♀)
Fourth nymphal	24/2♀♀ 25/1♀ 29/1♂ 31/1♀ 33/1♀ 34/2♂♂ 37/1♂ 39/1♂ 45/1♂ 48/1♀ 57/1♀ 58/2♂♂ 59/4(1♂ 3♀♀) 60/11(4♂♂ 7♀♀) 61/9(2♂♂ 7♀♀) 62/7(1♂ 6♀♀) 63/2(1♂ 1♀) 64/2(1♂ 1♀) 68/1♂	52 (19♂♂ 32♀♀) (1 died without molting)

from 10 to 34 days. In the third nymphal stage, molting began on the 15th day and was complete on the 41st. Forty-one ticks, 30 males and 11 females, reached the adult stage at this time. Fifty-two ticks remained for the fourth nymphal feeding. Molting began on the 24th day and was complete on the 68th. One tick died without molting. Of the 51 remaining ticks, 19 were males and 32 females—a total of 49 males and 43 females.

Summary of molting observations, Table 5. The duration of the larval stage for 262 ticks was 7 to 28 days; of the first nymphal stage for 217 ticks, 7 to 41 days; of the second nymphal stage for 209 ticks, 10 to 74 days; of the third nymphal stage for 192 ticks, 13 to 49 days; of the fourth nymphal stage for 98 ticks, 16 to 59 days; and for one tick in the fifth nymphal stage, 25 days. Of 198 ticks reared to adults, 18 males required 2 nymphal stages; 82 (45 ♂♂, 37 ♀♀), 3 nymphal stages, 97 (46 ♂♂, 51 ♀♀), 4 nymphal stages, and one female, 5 nymphal stages.

RELATION TO INFECTIOUS DISEASES

Relapsing fever. This species has not been definitely connected with human infection. However, it feeds readily on man and is the only species known in areas in which at least 17 cases have originated. Twelve specimens were collected from an endemic area in western Nevada. Spirochetes have been recovered from ticks collected in two areas in Wyoming, and one each in Montana, Utah, California, and Nevada. The Wyoming, Montana, and Utah strains have been studied in white mice, white rats, guinea pigs, and rhesus monkeys, the California strain only in white mice. Rhesus monkeys have not been shown to be susceptible. The other 3 hosts show typical relapses in varying degrees. In guinea pigs the reappearance of spirochetes in the peripheral blood is accompanied by clinical relapses.

Rocky Mountain spotted fever. O. parkeri has not been found infected in nature but ticks which have fed on infected guinea pigs readily produce the disease when later allowed to feed on fresh guinea pigs. Nymphs, males and females transmit the infecting agent. Two females have been shown to be infective 685 and 739 days, respectively, following the infective feeding in the second nymphal stage, and generation-to-generation transmission has been demonstrated.

American Q fever (Nine Mile fever). The causative organism (*Rickettsia diaporica*) survives and retains its virulence for many months in the tissues of successive stages of *O. parkeri,* but is not transmitted during the process of feeding. The tick excrement is infective.

Tularaemia. B. tularense survives and retains its virulence for at least 701 days, but is not transmitted during the process of feeding.

TABLE 5.—Ornithodoros parkeri. *Summary of molting observations. Figures outside parenthesis, 16–28 = limits of respective stages in days; figures in parenthesis, (41) = number of ticks in respective stage*

Stock	Larvae	1st nymphs	2d nymphs	3d nymphs	4th nymphs	5th nymphs	Adults
Montana	16–28(41)	14–41(33)	14–31(28)	15–32(19)	20–49(18)		19 16♂♂, 3♀♀
Wyoming	8–12(48)	13–18(35)	14–74(34)	16–49(34)	16–28(29)	25(1)	33 14♂♂, 19♀♀
California	10–13(59)	11–19(54)	12–23(54)	13–20(36)			54 30♂♂, 24♀♀
Utah	7–13(114)	7–12(95)	10–34(93)	15–21(93)	24–59(51)		92 49♂♂, 43♀♀
Summary	7–28(262)	7–41(217)	10–74(209)	13–49(182)	16–59(98)	25(1)	
Adults following respective molt ..			18♂♂	82(45♂♂, 37♀♀)	97(46♂♂, 51♀♀)	1♀	198 109♂♂, 89♀♀

CANNIBALISM

The phenomenon of one tick feeding on another of the same species has been termed cannibalism, which is obviously a misnomer as the tick host is neither *consumed* nor sufficiently harmed to prevent normal functions. The two following observations were made: August 30, 1939, a last stage nymph feeding on a guinea pig was punctured through the dorsum by a second tick which proceeded to completely engorge. The "host" tick molted normally to a female, copulated and oviposited; there were 198 eggs, 46 larvae. August 31, 1939, another last stage nymph was similarly punctured and the "host" tick again completely engorged. The latter molted normally to a female, copulated and oviposited. Eggs, 190; larvae, 82.

Similar observations on *O. turicata* were made by Wood (1912) and Francis (1938) and on *O. talaje* (unpublished notes, Davis, 1939b).

TABLE 6.—Ornithodoros parkeri: *oviposition data*

Female No.	Stock	Date of engorgement	Date of copulation	First eggs	First larvae	Total eggs	Total larvae
25	Wyoming	5-22-39	5–22-39	8–12–39	8–27–39	156	145
42	"	5–19–39	5–19–39	6–12–39	6–30–39	191	37
18	"	5–11–39	5–11–39	6– 4–39	6–21–39	186	10
48	"	11–14–39	11–14–39	1–16–40	2– 8–39	331	147
4	"	9– 7–39	9– 7–39	9–29–39	11–18–39	158	81
2	"	10–12–39	10–12–39	12–18–39	1–10–39	134	15
2	"	1–19–40	Unknown	2– 5–40	2–21–40	181	116
4	Utah	9–20–39	9–20–39	11–22–39	12–13–39	377	293
4	"	12–22–39	12–22–39	1–11–40	1–25–40	336	162
2	Nevada	11–30–39	Unknown	2– 2–40	2–19–40	171	104
2	Washington	11–15–39	11–16–39	3– 1–40	3–17–40	190	82
9	"	11–13–39	11–13–39	3–12–40	3–30–40	198	46
4	California	10–18–39	10–18–39	11– 4–39	11–26–39	276	71

OVIPOSITION (TABLE 6)

O. parkeri has not been observed to oviposit prior to copulation. Following engorgement and copulation, it oviposits less regularly than *turicata* under similar laboratory conditions. In Wyoming and Montana stock, which we have used most extensively, there is a marked tendency toward a rest period through the winter and early spring months. Table 6 shows the dates of engorgement and copulation, the appearance of the first eggs and of the first larvae, with the total number of eggs and larvae for each of 11 females. It includes data on Wyoming, Utah, Nevada, Washington, and California stock. In the case of the female from Utah, and one from Wyoming, data for 2 successive ovipositions are given. The shortest interval between engorgement and oviposition was 17 days and the longest 120. The largest number of eggs deposited at one oviposition was 377 and the average 222.

SUMMARY

Data on the distribution, type areas, habitats, and natural and experi-

mental hosts of the argasid tick, *Ornithodoros parkeri,* are given and observations on feeding habits, coxal fluid, longevity, copulation, molting, oviposition, cannibalism and its relation to relapsing fever, Rocky Mountain spotted fever, American Q fever and tularaemia are presented.

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A NEW SPECIES OF *ORNITHODOROS* TICK FROM NEVADA (ACARINA: IXODOIDEA)

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Ornithodoros cooleyi n. sp.

(Figs. 1–5)

Adult female. (Male not known.)

Body: Length 12 mm, width 7 mm. Ovate-oblong, somewhat narrowed and with margin rather sharply rounded anteriorly, hood not visible dorsally. Integument medium thick, sandy in color in the unengorged specimen. True discs absent, unmammillated tracts present, appearing as furrows in the unengorged tick. Numerous pit-like depressions present. Microscopically, integument irregularly mammillated, mammillae surrounded by sinuous grooves. Dorsally, sculpturing consists of rather shallow, bilaterally arranged depressions with a shallow median posterior groove, anterior marginal groove indistinct and broadly U-shaped. Margin of body rounded in unengorged specimen. Ventrally, integument similar to that of dorsum. Eyes lacking. Sub-coxal and supra-coxal folds not well marked. Supra-coxal folds extending anteriorly to form the margins of the hood, supra-coxal grooves extending laterally to coxae IV, expanding into triangular depressions surrounding the spiracles. Supra-coxal folds not diverging to form a notch in the lateral margins of the body. Sub-coxal folds originating at the base of coxae II expanding and extending to coxae IV. Median post-anal groove not well marked. Transverse pre-anal and post-anal grooves absent.

Anus: Ovate.

Spiracles: Located in triangular expansions of the supra-coxal grooves dorsal and slightly anterior to coxae IV. Spiracle circular, with a circular plate.

Hood: Large, broadly pointed anteriorly.

Capitulum: Placed in a poorly defined camerastome, with few hairs.

Hypostome: Slightly lanceolate, of fair size, apex emarginate. Dentition: Crowned with three or four semi-circular rows of minute teeth followed by two semi-circular rows of slightly larger teeth. Principal dentition consisting of two bilateral rows of five large teeth. Posterior to these a group of five smaller teeth.

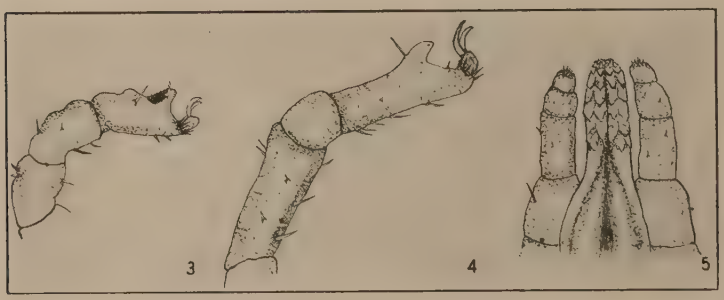
Palpi: Comparatively long and slender.

Legs: Slender, coxae progressively smaller from I to IV. Coxae II, III and IV contiguous; coxae I and II separated. Tarsus I equipped with three well defined diagnostic protuberances with a Haller's organ separating the distal two; claws surrounded by spines. Tarsus IV equipped with one well defined protuberance above the claws, claws surrounded by spines, one lanceolate spine present at the apex of the distal protuberance, numerous hairs present on the tarsus.

Locality: Taken near Rox, Lincoln County, Nevada.

Holotype: Deposited in the California Academy of Sciences, Golden Gate Park, San Francisco.

Received for publication, November 18, 1940.



Ornithodoros cooleyi n. sp. adult female

FIG. 1. Dorsal view. FIG. 2. Ventral view. FIG. 3. Tarsus of 1st leg. FIG. 4. Tarsus of 4th leg. FIG. 5. Hypostome and palpi.

THE RELATION OF PHYSID AND PLANORBID SNAILS TO THE LIFE CYCLE OF THE STRIGEID TREMATODE, *COTYLURUS FLABELLIFORMIS* (FAUST, 1917)[1]

W. W. Cort, Louis Olivier, and Sterling Brackett

The first account of any stage in the life cycle of the duck strigeid, *Cotylurus flabelliformis,* was given by Faust (1917a and b), who described as *Cercaria flabelliformis* a strigeid metacercaria which he found parasitic in rediae in *Physa gyrina* Say. These rediae also contained daughter rediae, and at the time Faust thought that they belonged to the life cycle of the strigeid. Hughes (1929) gave a more complete description of Faust's metacercaria and called it *Tetracotyle flabelliformis.* His material came from 9 specimens of *Lymnaea stagnalis perampla* Walker and from one specimen of *Helisoma campanulatum smithii* (Baker). He noted that in the planorbid some of the tetracotyles were inside the sporocysts of another trematode. Cort (1917) gave a preliminary description of a strigeid from a species of *Physa* and named it *Cercaria douglasi,* and Cort and Brooks (1928) identified as *C. douglasi* cercariae found in *Stagnicola emarginata angulata* (Sowerby), *Lymnaea stagnalis appressa* Say, *L. stagnalis perampla,* and *Physa parkeri* Currier. Their description was actually made from the cercariae obtained from three specimens of the varieties of *L. stagnalis.* Van Haitsma (1931) fed tetracotyles which he identified as *T. flabelliformis,* to domestic ducks and obtained adult strigeids belonging to the genus *Cotylurus.* The name of the species, therefore, became *C. flabelliformis* (Faust, 1917). Most of the tetracotyles used by Van Haitsma in his feeding experiments came from natural infections of lymnaeid snails although he also used a natural infection from *Helisoma trivolvis* (Say). In one of his experimental infections he utilized metacercariae which had developed in the varieties of *L. stagnalis* from experimental infections with the cercariae from these same hosts. It has recently been shown (Olivier and Cort, 1941) that the cercariae described by Cort (1917) and Cort and Brooks (1928) are actually two distinct species: *C. douglasi* Cort, 1917 (nec Cort and Brooks, 1928) which occurs only in *Physa* spp. and the cercaria of *C. flabelliformis* (= *C. douglasi* Cort and Brooks, 1928) which parasitizes only lymnaeids.

Received for publication, November 27, 1940.

[1] From the University of Michigan Biological Station and the Department of Helminthology, School of Hygiene and Public Health, the Johns Hopkins University. This paper is the third of a series on the researches on the second intermediate host relations of *Cotylurus flabelliformis* carried out in the Douglas Lake region beginning with the summer of 1932. The first paper in this series was by Winfield (1932) and the second by Nolf and Cort (1933).

In can be seen from the discussion given above, that the metacercarial stage of *C. flabelliformis* has been reported from snails belonging to three families, viz., LYMNAEIDAE, PHYSIDAE, and PLANORBIDAE. We found, however, in a series of preliminary experiments that in many cases development of the metacercariae could not be obtained in physid and planorbid snails exposed to the cercariae of *C. flabelliformis*, whereas lymnaeids were always successfully infected. It was decided, therefore, to carry out further experiments on the relations of these snails to the life cycle of this strigeid. In all, 13 experiments were performed in the summers of 1935, 1936, 1937, and 1940. The results, without exception, showed that physid and planorbid snails, negative for cercarial infections, were entirely unsuitable second intermediate hosts for the development of the metacercariae of *C. flabelliformis*. When, however, these snails were infected with the sporocysts and rediae of certain species of cercariae, the metacercariae of *C. flabelliformis* developed normally in them to the tetracotyle stage.

MATERIALS AND METHODS

Water containing cercariae of *C. flabelliformis* that had escaped from infected specimens of the varieties of *L. stagnalis* was poured into aquaria containing lymnaeid snails, for controls, and either physids or planorbids some of which were known to harbor infections of cercariae. Large numbers of the cercariae of *C. flabelliformis*, usually from several snails, were used, and frequently the exposure was continued for several days. In every experiment some of the snails died before the date of examination. These are entirely omitted from the protocols. Before the controls were placed in the experiments, examinations of some of the snails from the same collections were made for natural infections of tetracotyles; and before the experimental physids or planorbids were put in some of them were also examined.

The experiments were terminated from about 10 days to a month after the last exposure to the cercariae. At the time of examination each snail was carefully removed from its shell and all parts except the foot were teased apart as completely as possible. Since the metacercariae of *C. flabelliformis* normally develop in the hermaphroditic gland, the whole digestive gland in which this organ is embedded was always most carefully examined. The metacercariae were then counted and the stage of development recorded. The counts in the protocols are obviously always somewhat less than the actual numbers of the metacercariae present, since it is almost impossible to tease apart all the snail tissues to such a degree that all of them would be freed.

The developmental stages of the metacercariae of *C. flabelliformis* have never been described but the morphology of the same stages has been given for the closely related European species, *Cotylurus cornutus*

(Rud.) (cf. Szidat, 1924, and Wesenberg-Lund, 1934, Pl. XXV). When the cercaria penetrates into the snail it loses its tail and the body soon begins to enlarge. In about 10 days it has grown five to six times the length of the cercarial body and has become very broad and thick. The internal structures become very loosely organized and the space is occupied by stellate cells widely separated by large fluid-filled spaces. After maximum size is reached the tissues begin to be reorganized and condensed, the size decreases, and the hold-fast organ, the lateral cups, and other structures of the tetracotyle are formed. During this reorganization the cystogenous glands become prominent and a narrower hind-body is set off from the fore-body. The fully formed metacercaria (tetracotyle) is about one-third the length of the largest stage and is surrounded by a secreted cyst. The metacercariae recovered in our experiments were classified into three groups according to age, viz., (1) "developing" (all stages up to the largest size and those beginning to reorganize), (2) "pre-cysts" (those with a distinct hind-body but without any cyst wall), (3) "cysts" (those surrounded by a cyst wall).

Protocols for only eight of the thirteen experiments are included since five were unsatisfactory due to death of controls, lack of previous examination of controls or experimental snails for natural infection, or because of the death of too large a proportion of the experimental snails that harbored cercarial infections.

The following planorbid and physid snails were use in the experiments:

Helisoma trivolvis (Say)
H. campanulatum smithii (Baker)
H. antrosum percarinatum (Walker)
Planorbula armigera (Say)
Physa gyrina Say
P. parkeri Currier

The lymnaeid controls were:

Lymnaea stagnalis appressa Say
L. stagnalis parampla Walker
Stagnicola palustris elodes (Say)

The following larval trematode infections were found in the examinations of the experimental snails:

Cer. *Clinostomum marginatum* (Rud.) (cf. Hunter and Hunter, 1935; Krull, 1934a).

Cer. *Macroderoides typicus* (Winfield) (cf. McMullen, 1935).

Cer. *Petasiger nitidus* Linton (cf. Beaver, 1939).

Cer. *Triganodistomum mutabile* (Cort) (= *Cercariaeum mutabile* Cort, 1918) (cf. Wallace, 1939).

Cer. *Apatemon sphaerocephalus* (= *Cercaria burti* Miller, 1923) (cf. Willey and Rabinowitz, 1938).

Cercaria dohema Cort and Brackett, 1937.

Cer. *Psilostomum ondatrae* Price (cf. Beaver, 1939).

Cer. *Alaria intermedia* (Olivier and Odlaug, 1938) (cf. Odlaug, 1940).

Cercaria physae Cort and Brooks, 1928.

Cer. *Uvulifer ambloplitis* (Hughes) (= *Cercaria bessiae* Cort and Brooks, 1928) (cf. Krull, 1934 b; Hunter and Hunter, 1935).

Cercaria elephantis Cort, 1917.

PROTOCOLS OF EXPERIMENTS IN WHICH SNAILS OF THE FAMILIES PLANORBIDAE AND PHYSIDAE WERE EXPOSED TO THE CERCARIAE OF *Cotylurus flabelliformis*

EXPERIMENT 1

Experimental snails: *H. trivolvis.*
Examination for natural infections: (none made)
Exposed to cercariae of *C. flabelliformis,* 6/23–7/28/37.
23 surviving snails examined, 7/28/37.
Results of examination:
20 without cercarial infection:
14 had no metacercariae.
6 had a total of 15 metacercariae (4 cysts, 2 pre-cysts, 9 developing).
2 with cer. *C. marginatum:*
188 and 64 metacercariae (52 cysts, 200 pre-cysts and developing).
1 with cer. *M. typicus:*
162 metacercariae (62 cysts, 100 developing).
Control snails: *L. stagnalis appressa* and *L. stagnalis perampla.*
Examination for natural infection:
20 without cercarial infection:
17 had no metacercariae
3 had a total of 4 metacercariae (cysts)
Examination for experimental infection:
8 without cercarial infection:
68–779, ave. 519 metacercariae (18% cysts, 1% pre-cysts, 81% developing).
Comment: One of the controls had an infection of the cercaria of *C. flabelliformis* which came to maturity during the course of the experiment. This accounts for the heavy metacercarial infections and for the presence of all stages of development.

EXPERIMENT 2

Experiment snails: *H. campanulatum smithii.*
Examination for natural infection: (none made)
Exposed to cercariae of *C. flabelliformis,* 6/23–7/2/37.
12 surviving snails examined, 7/13/37.
Results of examination:
5 without cercarial infection:
2 had no metacercariae.
3 had a total of 6 metacercariae (2 cysts, 4 developing).
4 with cer. *M. typicus:*
7, 9, 27, and 37 metacercariae (8 cysts, 35 pre-cysts, 32 developing).
1 with cer. *C. marginatum:*
3 metacercariae (cysts).
1 with cer. *P. nitidus:*
17 metacercariae (3 pre-cysts, 14 developing).

1 with both cer. *M. typicus* and cer. *U. ambloplitis*:
19 metacercariae (pre-cysts).
Control snails: *L. stagnalis appressa* and *L. stagnalis peramplа*.
Examination for natural infection: (same as experiment 1).
Examination for experimental infection:
7 without cercarial infection:
26–211, ave. 105 metacercariae (1% cysts, 28% pre-cysts, 71% developing).

EXPERIMENT 3

Experimental snails: *H. antrosum percarinatum*.
Examination for natural infection:
14 without cercarial infection:
no metacercariae.
5 with cer. *T. mutabile*:
no metacercariae.
1 with *C. dohema*:
no metacercariae.
Exposed to cercariae of *C. flabelliformis*, 8/1–3/40.
41 surviving snails examined, 8/11–13/40.
Results of examination:
25 without cercarial infection:
no metacercariae.
10 with cer. *T. mutabile*:
11–153, ave. 88 metacercariae (about 40 cysts, a number of pre-cysts, majority developing).
5 with cer. *A. sphaerocephalus*:
55–218, ave. 124 metacercariae (about same stages as in snails infected with cer. *T. mutabile*).
1 with *C. dohema*:
44 metacercariae (a few pre-cysts, most developing).
Control snails: *S. palustris elodes*.
Examination for natural infection:
14 without cercarial infection:
no metacercariae.
Examination for experimental infection:
9 without cercarial infection:
20–268, ave. 156 metacercariae (all developing).
Comment: The controls from all the 1940 series of experiments were specimens of *S. palustris elodes* from the same collection. Therefore, the examinations for natural infections of controls will be the same in all.

EXPERIMENT 4

Experimental snails: *H. antrosum percarinatum*.
Examination for natural infection:
5 without cercarial infection:
no metacercariae.
1 with cer. *P. ondatrae*:
no metacercariae.
1 with unidentified xiphidio-cercariae:
no metacercariae.
Exposed to cercariae of *C. flabelliformis*, 8/12–20/36.
59 surviving snails examined, 8/28–29/36.
Results of examination:
47 without cercarial infection:
no metacercariae.

4 with cer. *A. sphaerocephalus:*
538–751, ave. 631 metacercariae (3 pre-cysts, all the rest developing).
4 with cer. *P. ondatrae:*
46–410, ave. 216 metacercariae (all developing).
2 with cer. *T. mutabile:*
0 and 30 metacercariae (developing).
2 with cer. *M. typicus:*
5 and 142 metacercariae (developing).

Control snails: none used.

EXPERIMENT 5

Experimental snails: *P. armigera.*
Examination for natural infection:
16 without cercarial infection:
no metacercariae.
Exposed to cercariae of *C. flabelliformis,* 7/21–24/40.
76 surviving snails examined, 8/10/40.
Results of examination:
72 without cercarial infection:
no metacercariae.
2 with unidentified xiphidio-cercaria:
79 and 249 metacercariae (mostly cysts, a few pre-cysts and developing).
2 with cer. *A. intermedia:*
224 and 298 metacercariae (a few cysts and pre-cysts, most developing).

Control snails: *S. palustris elodes.*
Examination for natural infection:
(same as experiment 3).
Examination for experimental infection:
6 without cercarial infection:
126–383, ave. 236 metacercariae (1% cysts, 6% pre-cysts, 93% developing).

EXPERIMENT 6

Experimental snails: *P. gyrina.*
Examination for natural infection:
13 without cercarial infection:
10 had no metacercariae.
3 had a total of 16 metacercariae (10 pre-cysts, 6 developing).
9 with *C. physae:*
no metacercariae.
3 with echinostome cercaria:
2 had no metacercariae.
1 had 1 metacercaria (pre-cyst).
Exposed to cercariae of *C. flabelliformis,* 7/25–29/40.
82 surviving snails examined, 8/12–13/40.
Results of examination:
36 without cercarial infection:
21 had no metacercariae.
15 had a total of 44 metacercariae (37 cysts, 7 pre-cysts).
27 with *C. physae:*
5–452, ave. 159 metacercariae (62% cysts, 33% pre-cysts, 5% developing).
12 with echinostome cercaria:
9–459, ave. 86 metacercariae (almost all encysted).
4 with reniferid cercaria:
0, 122, 217, and 265 metacercariae (96% cysts, 4% pre-cysts).

3 with cer. *A. sphaerocephalus:*
20, 80, and 217 metacercariae (96% cysts, 4% pre-cysts).
Control snails: *S. palustris elodes.*
Examination for natural infection:
[illegible] same as experiment 1.
Examination for experimental infection:
2 without cercarial infection:
31 and 224 metacercariae (3% pre-cysts, 97% developing).

Comment.—The natural infection of the experimental snails was probably the metacercarial stage of *C. douglasi* (see discussion). *C. douglasi* was found in the summer of 1967 in one specimen of *P. parkeri* from the locality where the experimental snails used in experiment 6 were collected.

The echinostome cercariae in this experiment and experiment 6 probably belonged either to *Echinoparyphium recurvatum* or *Echinoparyphium recurvatum*, the cercariae of which are difficult to distinguish and which are both found in the species of *Physa* in the region.

The rendered cercariae probably belonged to one of the two species for which the life cycles were described by Talbot (1933). As noted by this author it is almost impossible to make accurate identifications of these species from the cercaria only.

EXPERIMENT 7

Experimental snails: *P. gyrina.*
Examination for natural infection:
13 without cercarial infection:
no metacercariae.
Exposed to cercariae of *C. flabelliformis*, 7/30/40.
27 surviving snails examined, 8/14/40.
Results of examination:
25 without cercarial infection:
no metacercariae.
1 with *C. physae:*
346 metacercariae (all cysts).
1 with unidentified xiphidio-cercaria:
46 metacercariae (all cysts).
Control snails: *S. palustris elodes.*
Examination for natural infection:
same as experiment 5.
Examination for experimental infection:
5 without cercarial infection:
13–185, ave. 96 metacercariae (3% cysts, 73% pre-cysts, 24% developing).

EXPERIMENT 8

Experimental snails: *P. parkeri* (large juveniles).
Examination for natural infection:
5 without cercarial infection:
4 had no metacercariae.
1 had 3 metacercariae (pre-cysts) (probably *C. douglasi*).
2 with *C. physae:*
no metacercariae.
3 with echinostome cercaria:
no metacercariae.
Exposed to cercariae of *C. flabelliformis*, 7/31–8/1/40.
37 surviving snails examined, 8/14/40.
Results of examination:
29 without cercarial infection:
26 had no metacercariae.
3 had 4 metacercariae (cysts) (probably *C. douglasi*).

27 with echinostome cercaria:
1 had no metacercariae.
26 had 1–152, ave. 71 metacercariae (99% cysts, 1% pre-cysts).
1 with *C. physae:*
109 metacercariae (cysts).
Control snails: *S. palustris elodes.*
Examination for natural infection:
(same as experiment 3).
Examination for experimental infection:
5 without cercarial infection:
7–92, ave. 38 metacercariae (37% cysts, 20% pre-cysts, 43% developing).

RESULTS OF EXAMINATION OF *H. trivolvis* NATURALLY EXPOSED TO INFECTION WITH THE CERCARIAE OF *C. flabelliformis*

A collection of *Helisoma trivolvis* was made during the summer of 1940 from an area on Douglas Lake where large numbers of the varieties of *L. stagnalis* lived in the same environment. Some of these lymnaeids were infected with the cercaria of *C. flabelliformis* since six specimens infected with this species appeared in about 300 snails examined. It seemed evident, therefore, that the snails in this area were being exposed to infection with this cercaria.

RESULTS OF EXAMINATION OF *H. trivolvis* (128 SPECIMENS OF LARGE AND MEDIUM SIZED ADULTS) FOR NATURAL INFECTIONS WITH METACERCARIAE OF *C. flabelliformis*

106 without cercarial infection:
no metacercariae.
12 with cer. *C. marginatum:*
2 had no metacercariae.
10 had 1–20, ave. 8 metacercariae.
4 with cer. *M. typicus:*
0, 29, 87, and 131 metacercariae.
3 with cer. *A. sphaerocephalus:*
26, 48, 434 metacercariae.
1 with cer. *U. ambloplitis:*
38 metacercariae.
1 with *C. elephantis:*
518 metacercariae.
1 with immature stylet:
no metacercariae.

Comment: Most of these metacercariae were fully developed tetracotyles, but there were also small numbers in all stages of development.

DISCUSSION

The data given above indicate that four species of planorbid and two of physid snails are unsuitable as second intermediate hosts for *C. flabelliformis* unless they harbor infections with certain species of cercariae. It is also evident from the experiments that the varieties of *L. stagnalis* and *S. palustris elodes* are normal second intermediate hosts for this species. In fact we have found that the metacercariae of this strigeid

will develop in all the species of lymnaeid snails found in the Douglas Lake region, whether or not they are infected with larval trematodes.[2] Snails of the family LYMNAEIDAE, therefore, can be considered as the normal second intermediate hosts of *C. flabelliformis.*

When the development does occur in the physid and planorbid snails it appears to be entirely normal. In fact, as mentioned earlier, Van Haitsma obtained adults of *C. flabelliformis* by feeding tetracotyles from a planorbid snail to a domestic duck. When physid and planorbid snails are exposed to the cercariae of *C. flabelliformis,* penetration occurs whether or not the snails are infected with species of cercariae. In those that are uninfected the cercarial bodies do not develop and soon die. On one occasion three juveniles of *P. parkeri,* one adult of *H. antrosum percarinatum,* and one juvenile of *Stagnicola emarginata angulata* were placed in a small dish with large numbers of the cercariae of *C. flabelliformis* and were observed under a binocular microscope. The cercariae were seen to penetrate readily into all five snails and in a few minutes numerous detached tails were found in the dish. Four days later when these snails were examined numerous dead cercarial bodies were found in the specimens of *Physa* and *Helisoma.* The specimen of *S. emarginata angulata* contained numerous living cercarial bodies. Also, in one of the experiments with *P. gyrina,* presented above, it was observed that the snails showed great irritation after unusually large numbers of cercariae of *C. flabelliformis* had been poured into the aquarium. They reacted by moving the shell rapidly back and forth. This same reaction frequently occurs when lymnaeid snails are exposed to large numbers of these cercariae, and seems to result from the irritation produced by their penetration.

In the physid and planorbid snails that had been successfully infected with larvae of *C. flabelliformis,* metacercariae were always found inside the sporocysts and rediae. When sporocysts were present a large proportion of the metacercariae were also found free in the dish probably because the sporocysts were always broken up by the teasing of the digestive gland. In the infections with rediae (*C. marginatum, T. mutabile, P. ondatrae,* and the echinostomes) only a small proportion of the metacercariae were ever found outside the rediae. This seemed to be due to the fact that few of the rediae were broken in the teasing of the preparations. Also of interest in this connection is the specimen of *P. gyrina* in experiment 7, infected with an unidentified xiphidiocercaria. In this snail all of the sporocysts were in a clump well in front of the digestive gland. Examination showed that all the metacercariae were in these sporocysts and not a single one was found elsewhere. From this evidence we conclude that in these abnormal hosts the metacercariae of *C. flabelliformis*

[2] Further discussion of these experiments will be reserved for a later paper.

develop inside the sporocysts and rediae and, therefore, exhibit a form of hyperparasitism.

The metacercariae of *C. flabelliformis* may also be hyperparasites in normal hosts that harbor infections with various species of cercariae. In fact in our experimental infections of lymnaeid snails with this cercaria we have found the metacercariae inside sporocysts and rediae whenever they were present. Also, in the literature on larval trematodes, strigeid metacercariae, especially the fully developed tetracotyles, have frequently been reported and figured inside sporocysts and rediae. Such observations are recorded in some of the earliest studies on trematode development.[3]

Another significant point is that the development of the metacercarial stage inside sporocysts and rediae in an abnormal host is completed more rapidly than in the hermaphroditic gland of a normal host without cercarial infection. While this is suggested in almost all of the experiments, it is shown most clearly in experiments 7 and 8, in which most of the metacercariae were encysted after two weeks in the physids harboring rediae or sporocysts while in the controls the majority were still in the "developing" or "pre-cyst" stages. In normal hosts also, the development of the metacercariae is more rapid inside the sporocysts and rediae of infected individuals than in the hermaphroditic gland of those harboring no cercarial infections.

A further point shown in the protocols is that a few metacercariae, including mature tetracotyles, were present in several physid and planorbid snails that harbored neither rediae nor sporocysts. Those found in the physids were probably the metacercariae of *C. douglasi,* which is present in species of *Physa* in this region and which uses these same snails as normal second intermediate hosts. The metacercariae of *C. douglasi* are very similar to those of *C. flabelliformis.* It is possible that the metacercariae found in experiments 1 and 2 in a few specimens of *Helisoma* which harbored no cercarial infections were really those of *C. flabelliformis.* Miss Ruby Wortham, who has examined large numbers of *H. trivolvis* from an area where this strigeid is present, informed us that in a few cases small numbers of tetracotyles were found in snails that did not harbor cercarial infections. Since it is impossible to tell from experiments 1 and 2 whether the metacercariae came from natural or experimental infections, the possibility is still present, however, that they were not those of *C. flabelliformis* but of some other closely related strigeid.

It is difficult to understand just why the presence of sporocysts and rediae in physid and planorbid snails makes possible the development of the metacercariae of *C. flabelliformis.* When these abnormal hosts are

[3] A discussion of these early findings is given by Wesenberg-Lund (1934, pp. 141–142).

uninfected with sporocysts or rediae the failure of the metacercariae to develop may be due either to an immune reaction that kills the cercariae after they penetrate or to the absence of suitable conditions for their persistence and development; their death soon after penetration suggests a host reaction but it is possible that suitable food is not available. In the abnormal hosts already harboring cercarial infections the penetrating cercariae of *C. flabelliformis* apparently escape those unfavorable conditions by entering the sporocysts or rediae. Here, as hyperparasites, they find protection from adverse host reactions, entirely escape unfavorable conditions in the tissues, and obtain substances for food that have been prepared by the sporocysts or rediae for their developing progeny. It seems probable, since they develop more rapidly under these conditions than in the tissues of the normal host, that improved nutrition is an important factor in their successful development.

SUMMARY

Various species of the family LYMNAEIDAE are the usual second intermediate hosts of the duck strigeid, *Cotylurus flabelliformis*. The cercariae of this strigeid, however, penetrate into planorbid and physid snails, but develop only in those individuals that harbor sporocysts and rediae of larval trematodes; in uninfected snails they do not develop and soon die. The development of the metacercariae in such abnormal hosts takes place inside the sporocysts and rediae where the tetracotyle stage is reached more quickly than in their normal environment which is the hermaphroditic gland of lymnaeid species. It is suggested that when the cercarial bodies penetrate into sporocysts or rediae, they are protected from any immune reaction of the abnormal host, and as hyperparasites, are able to utilize very effectively in their nutrition the food material which these trematode parasites have absorbed from the tissues of the snail host and have prepared for the nourishment of their own developing progeny. Since the development of the metacercariae inside the sporocysts and rediae in the abnormal host appears to be entirely normal, it seems very probable that physid and planorbid snails, which harbor cercarial infections, may have a regular part as second intermediate hosts in carrying out the life cycle of *C. flabelliformis*.

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SPICULE LENGTH IN *COOPERIA CURTICEI* AS A MEASURE OF FAVORABLE INTESTINAL ENVIRONMENT FOR THIS INTESTINAL NEMATODE OF SHEEP

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From a study of the distribution of trichostrongylids in the small intestine of the sheep Tetley (1937) concluded that their particular location was determined by the rate of response of incoming larvae to conditions within the alimentary canal and that, following taking up their station, virile subsequent stages of the parasites migrated to an inappreciable degree. It was further concluded that parasites were able to survive in a wider range of area and physiological condition of the small intestine than they actually invaded.

In pursuance of this investigation it was considered that comparative study of parasites recovered from different intestinal regions in a given host would add to information on their relation to the environment within the given host. Thus comparison of the extent of their growth in various parts of the intestine by indicating something of the relative suitability of these regions for the parasites would be a step towards understanding how parasites come to select their chosen site of establishment.

Length of spicules has been taken as a suitable index of growth, for the ease with which these structures may be measured with accuracy, and the fact that they are not affected by the various treatments preparatory to measurement, rendered them technically convenient to handle. *Cooperia curticei* was chosen as a favorable species for investigation. Its extended range of infection in the jejunum and the constancy of its frequency distribution made it likely that comparison could be carried out between sections of a population between which there had been a minimum of intermingling.

MATERIAL AND METHODS

Material was collected from infections acquired in the field by Romney cross bred sheep at Palmerston North, New Zealand. Ingesta containing worms were removed from sections of the small intestine with as little disturbance of those adjoining as possible. Worms were preserved in glycerin-alcohol. Measurements were made of glycerin jelly mounts using an ocular micrometer.

RESULTS

The results pertaining to three sheep are contained in Tables 1, 2, and 3. For convenience of tabulation the data have been grouped in 5

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TABLE 1.—*The spicule measurements of* Cooperia curticei *from three sections of the small intestine as measured from the anterior end (Sheep I)*

Class (microns)	Frequencies of spicule lengths, in micron classes, for intestinal sections:			
	10–15 feet	16–20 feet	21–25 feet	Total, 10–25 feet
111–115	2	–	1	3
116–120	30	6	16	52
121–125	45	–	21	66
126–130	76	8	33	117
131–135	65	15	29	109
136–140	26	2	19	47
141–145	14	1	7	22
146–150	2	–	2	4
Total spicules measured	260	32	128	420
Mean length ± P.E.	128.7 ± 0.3	129.5 ± 0.8	129.0 ± 0.4	128.9 ± 0.2
Standard deviation	6.9	6.7	7.1	6.9

TABLE 2.—*The spicule measurements of* Cooperia curticei *from five sections of the small intestine as measured from the anterior end (Sheep II)*

Class (microns)	Frequencies of spicule lengths, in micron classes, for intestinal sections:					
	1–5 feet	6–10 feet	11–15 feet	16–20 feet	21–25 feet	Total, 1–25 feet
121–125	–	4	2	7	6	19
126–130	3	14	2	13	12	44
131–135	19	34	2	19	22	96
136–140	5	16	8	16	18	63
141–145	4	11	10	6	6	37
146–150	3	3	2	5	–	13
151–155	–	–	–	–	4	4
156–160	–	–	–	2	2	4
161–165	–	–	2	–	–	2
Total spicules measured	34	82	28	68	70	282
Mean length ± P.E.	135.7 ± 0.6	133.9 ± 0.4	139.5 ± 1.1	134.2 ± 0.6	135.0 ± 0.4	134.9 ± 0.3
Standard deviation	5.1	5.8	8.4	7.7	5.5	7.6

TABLE 3.—*The spicule measurements of* Cooperia curticei *from five sections of the small intestine as measured from the anterior end (Sheep III)*

Class	Frequencies of spicule lengths, in micron classes, for intestinal sections:					
	8–10 feet	11–12 feet	13–14 feet	17–18 feet	23–24 feet	Total, 8–24 feet
121–125	–	3	2	2	1	8
126–130	2	3	3	2	5	15
131–135	16	6	25	5	15	67
136–140	10	5	19	5	11	50
141–145	5	4	9	2	13	33
146–150	1	3	2	–	1	7
151–155	–	–	–	–	2	2
Total spicules measured	34	24	60	16	48	182
Mean length ± P.E.	135.5 ± 0.5	135.3 ± 1.0	135.7 ± 0.4	133.7 ± 1.0	136.5 ± 0.6	135.6 ± 0.3
Standard deviation	4.6	7.4	4.8	5.9	6.4	5.8

micron classes. Computations have been made, however, on the basis of one micron classes in which form the data had been originally collected.

DISCUSSION

The material having been obtained from infection acquired in the field, it is not possible to determine precisely the factors, specific and non-specific, that might have operated to cause particular portions of the worm population to inhabit particular portions of the small intestine. This would be difficult were the material from pure infections of one age. It is concluded that the normal frequency distribution found in the data for spicule lengths for populations within the various regions of intestinal residence is indicative of the uniformity of the growth conditions for the majority of worms within those particular areas. If there had been a selective action, by factors imposed on the worms in the course of their establishment in those regions of the intestine which they were found to be inhabiting, it would be expected that some distinctive form of distribution would have resulted and would have been recognizable. This assumption underlies the possibility that comparison of spicule lengths of the sub-populations of the various areas would indicate the relative favorability of the growth conditions for spicules within those areas. The fact that differences as between different sheep (see below) are thus recognizable lends support to the assumption, that differences within a given sheep would similarly be apparent.

From Tables 1, 2 and 3 it can be seen that the means for spicule length of *C. curticei* for the different intestinal regions in which the parasite established itself, actually exhibited variance to only an insignificant degree. Between sheep I and, on the other hand, sheep II and III the means for the total numbers of spicules measured, differed materially, and to a statistically significant degree. The conclusion is therefore drawn that the sum of the effects of the various influencing factors differed to a greater extent between sheep than between regions of residence in the same sheep. This is another way of saying that from the point of view of growth to maturity the regions for which data were available displayed no significant differences in favorability.

SUMMARY

In material from field-acquired infections comparison was made of the lengths of spicules of *Cooperia curticei* found in different parts of the small intestine of sheep. While inter-host differences in the mean lengths of spicules were statistically demonstrable, intra-host differences as between different levels of intestinal residence, were not apparent. It is concluded that in regard to favorability for growth all parts of the whole intestinal area in which the worms established themselves, and

from which they were selectively measured for spicule length, were equally acceptable to this nematode parasite.

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HAEMONCHUS CONTORTUS EGGS: COMPARISON OF THOSE IN UTERO WITH THOSE RECOVERED FROM FECES, AND A STATISTICAL METHOD FOR IDENTIFYING *H. CONTORTUS* EGGS IN MIXED INFECTIONS

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By confining attention to the cores of the frequency distribution of lengths plotted against widths, the author (1935) found that it was possible to distinguish between the greater number of eggs of *Nematodirus filicollis* and *N. spathiger*. Shorb (1939, 1940) has applied the method to eggs dissected from worms of other species of trichostrongylids found in ruminants.

For the reason that normal practice requires identification of eggs voided in feces it seemed desirable that comparison should be made between eggs dissected from females and those which had been recovered from feces by ordinary isolation techniques. Tetley (1941) found that among species of *Nematodirus* eggs obtained from feces and measured in the course of making dilution counts by the Stoll method no change in form from that of eggs freshly dissected from females could be detected. In the present account the, by contrast, thin-shelled eggs of *Haemonchus contortus* are considered.

METHODS AND MATERIALS

An ocular micrometer was used in measuring eggs. Dissections of eggs from females and measurements were carried out on fresh material, within a few hours of the death of the host. Eggs of fecal origin were measured partly in the course of making dilution counts by the Stoll method and on other occasions following isolation by a centrifugal method that involved flotation in saturated salt solution.

With the exception of the data from sheep VI and VII of a Dorset-Shropshire cross, located at the Rockefeller Institute for Medical Research, Princeton, New Jersey, the bulk of the data was derived from Romney cross sheep at Palmerston North, New Zealand.

Sheep I: Slaughtering date: May 20, 1937. Age: Eight to nine months. Ninety-seven segmented eggs were dissected from 8 females of *H. contortus* and measured. The results are contained in Table 1. Eggs

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were also dissected from females of a number of other species present. The range of distribution of all species has been plotted in Fig. 1.

Sheep II: Slaughtering date: March 11, 1936. Age: Seven to eight months. One hundred and eighty-four segmented eggs were dissected from 4 worms taken at random, and measured. Results are contained in Table 1.

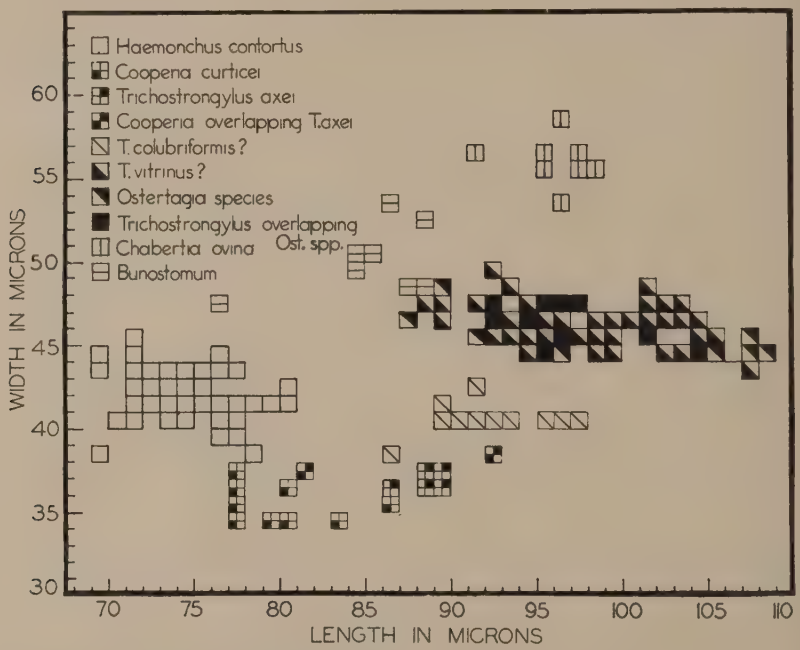


FIG. 1. Range of measurements of eggs dissected from *Haemonchus contortus, Cooperia curticei, Trichostrongylus axei, T. colubriformis* (?), *T. vitrinus* (?), *Ostertagia* species, *Chabertia ovina* and *Bunostomum trigonocephalum* from sheep. For details of the *Haemonchus* data see Table 1. The relative disposition of the specific groupings approximates to that found among eggs from feces in mixed infections (Fig. 8).

Sheep III: Slaughtering date: March 16, 1936. Age: Seven to eight months. Two hundred and thirty-four segmented eggs were dissected from 8 worms selected at random, and measured. Results are contained in Table 1.

Sheep IV: Slaughtering date: March 5, 1932. Age: Seven to eight months. From 4 females of *Haemonchus contortus* taken at random, 77 segmented eggs were dissected and measured. The results are contained in Table 1 and Fig. 2.

Sheep V: Slaughtering date: May 26, 1935. Age: Five years. At

TABLE 1.—*Statistical analysis of data on the dimensions of eggs of* Haemonchus contortus. *For method of computation of range of fractions of the egg populations see text*

Sheep	Source of eggs : worm	Number of eggs measured	Mean		Size range of eggs		
			Length μ	Width μ	100 per cent (observed) μ	91.2 per cent (computed) μ	25 per cent (computed) μ
I	A	14	76.2	42.9	67–81 by 38–45	70–80 by 38–45	73–77 by 40–42
	B	30	75.0	41.1			
	C	22	78.1	39.4			
	D	11	70.3	43.1			
	Total A–D	77	75.12 ± 0.19	41.28 ± 0.14			
			S.D. 2.44	S.D 1.78			
			C.V. 3.3%	C.V. 4.3%			
II	E	60	78.9	40.3	66–87 by 38–44	70–83 by 38–42	74–79 by 39–41
	F	45	74.4	40.9			
	G	34	74.3	40.0			
	H	45	77.7	39.7			
	Total E–H	184	76.68 ± 0.16	40.21 ± 0.06			
			S.D. 3.29	S.D. 1.13			
			C.V. 4.3%	C.V. 2.8%			
III	I	55	74.6	42.7	69–86 by 39–46	70–84 by 39–44	74–79 by 41–43
	J	45	77.8	40.9			
	K	10	77.8	41.7			
	L	13	73.2	41.2			
	M	16	72.1	41.3			
	N	15	76.3	40.8			
	O	40	79.4	41.6			
	P	40	79.5	41.4			
	Total I–P	234	76.84 ± 0.16	41.58 ± 0.06			
			S.D. 3.57	S.D. 1.37			
			C.V. 4.6%	C.V. 3.3%			
IV	Q	14	78.0	42.4	70–81 by 39–47	69–80 by 39–45	73–76 by 41–43
	R	17	75.0	42.1			
	S	15	72.6	41.8			
	T	12	77.5	43.9			
	U	10	76.6	40.4			
	V	9	75.3	40.4			
	W	10	72.8	41.2			
	X	10	71.6	44.1			
	Total Q–X	97	74.54 ± 0.18	42.09 ± 0.11			
			S.D. 2.62	S.D. 1.51			
			C.V. 3.5%	C.V. 3.6%			
V	Y	66	77.7	45.3	63–93 by 31–48	72–85 by 38–46	76–80 by 41–43
	Z	30	78.1	41.8			
	AA	25	78.9	34.6			
	BB	30	80.1	42.5			
	Total Y–BB	151	78.36 ± 0.19	41.92 ± 0.11			
			S.D. 3.13	S.D. 1.84			
			C.V. 4.0%	C.V. 4.4%			
I–V	Total 28 worms	743	76.61 ± 0.09	41.25 ± 0.05	66–87 by 34–47	69–84 by 37–46	74–79 by 40–43
			S.D. 3.78	2.20			
			C.V. 4.9%	5.3%			
V	Feces	111	78.49 ± 0.19	44.90 ± 0.11	72–89 by 39–51	72–85 by 41–48	76–81 by 44–46
			S.D. 3.11	S.D. 1.72			
			C.V. 4.0%	C.V. 3.8%			
VI	Feces	60	70.77 ± 0.32	45.15 ± 0.14	62–80 by 42–50	63–78 by 42–48	68–73 by 44–46
			S.D 3.69	S.D. 1.66			
			C.V. 5.2%	C.V. 3.7%			
VII	Feces	60	73.72 ± 0.33	45.48 ± 0.17	65–83 by 42–50	66–81 by 42–49	71–76 by 44–47
			S.D 3.82	S.D. 1.94			
			C.V. 5.2%	C.V. 4.3%			

S.D. = Standard deviation.
C.V. = Coefficient of variation.

autopsy 750 females of *H. contortus* and a few individuals of *Ostertagia circumcincta, O. trifurcata* and *Trichostrongylus axei* were found. One hundred and twenty-seven eggs were broken out from 4 females of *H. contortus* taken at random, and measured. These results are summarized in Table 1. Measurements of eggs found in feces in the course of making dilution counts by the Stoll method were made at intervals over a period of approximately three years. The aggregated results from this latter source are plotted in Fig. 4. Measurements of fecal eggs made at the time of autopsy have been replotted in Fig. 3.

Sheep VI and VII: Date of fecal sampling: May 7, 1940. Age of sheep: Over one year. Pure infections of *H. contortus* were present in these two animals. Eggs were secured from freshly collected feces by means of a centrifugal flotation method, using saturated salt solution. Measurements were made of 60 eggs from each animal and these are plotted in Figs. 5 and 6 respectively.

Flock of fifty sheep: Dates of fecal sampling: Between March 7,1935, and January 21, 1936. Age of sheep: Five years and older. Measurements of eggs were obtained in the course of making counts by the Stoll dilution technique of eggs in periodically collected samples of feces from a flock of 50 sheep. These are plotted in Fig. 8.

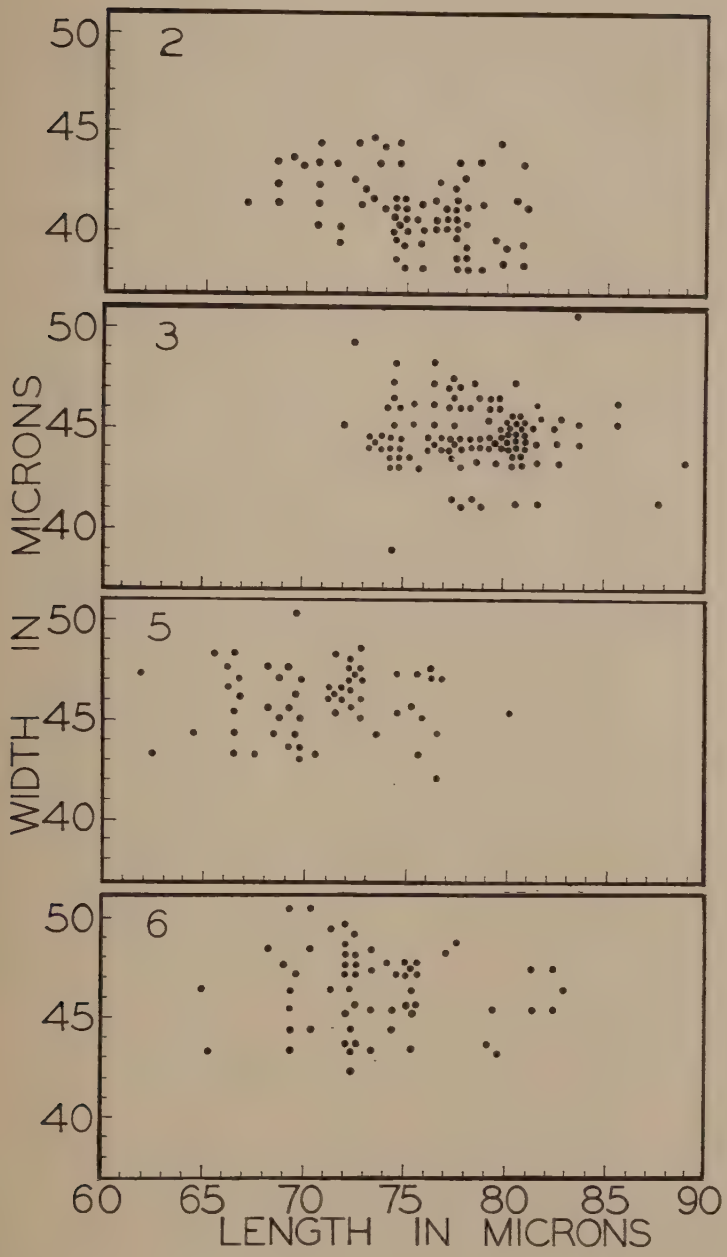
ANALYSIS OF DATA

A statistical analysis of the data has been made. This is set out in Table 1. Means for aggregated results were computed from the totals of eggs from sheep, not from the means for individual worms. The populations of eggs have been regarded as having a bivariate normal frequency distribution and accordingly formulae relating to such distributions have been applied to the data. The small amount of negative correlation between the two axes has been ignored. Statistical ranges of means plus and minus respectively 0.6745 and twice the standard deviations which in a universe would enclose respectively 25 per cent and 91.2 per cent of the population have been calculated for the data, to the nearest micron.

FIG. 2. Length plotted against width of eggs dissected from *Haemonchus contortus* from sheep IV (see Table 1). The bivariate normal frequency distribution is typical for eggs dissected from worms from other sheep (see Table I and Fig. 7). Width of eggs dissected from worms tends to be less than that of eggs obtained from feces (see Figs. 3, 5, and 6).

FIG. 3. Length plotted against width of eggs of *Haemonchus contortus* obtained from feces of sheep V at autopsy (see Table 1). The grouping persisted for eggs from the feces of the same animal over three years (see Fig. 4) and coincided with a group among the measurements of eggs from feces in 50 mixed infections (see Fig. 8).

FIGS. 5 and 6. Length plotted against width of eggs of *Haemonchus contortus* from the feces of sheep VI and VII, respectively. Width is similar to that of eggs from the feces of sheep V (Fig. 3) though length is less.


2
3
5
6
50
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WIDTH IN MICRONS
60
65
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75
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85
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LENGTH IN MICRONS

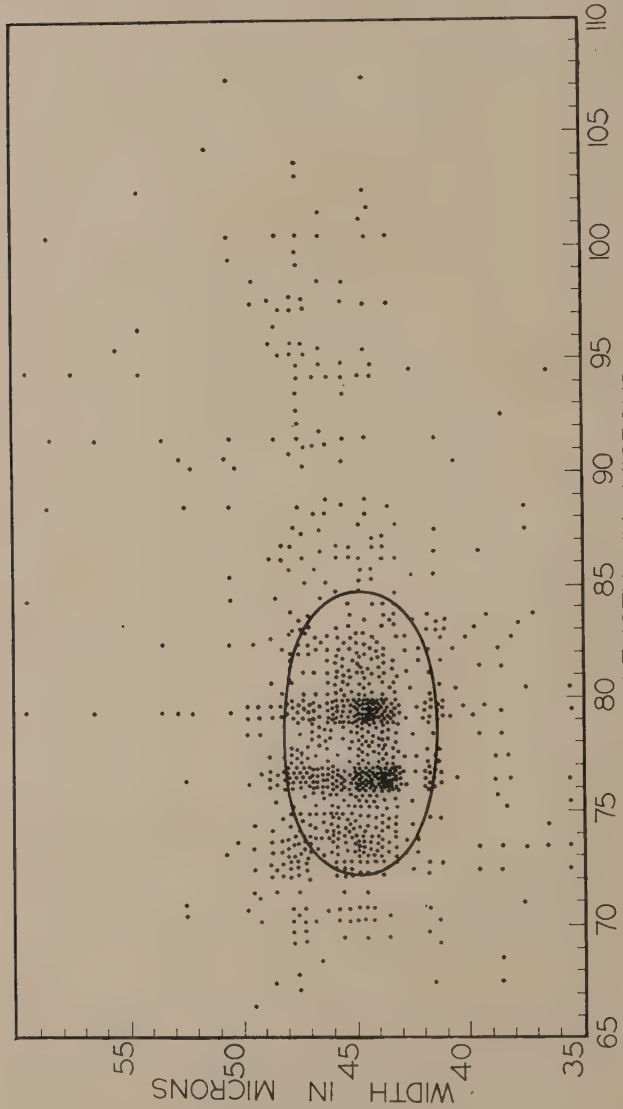

FIG. 4. Length plotted against width of eggs from feces of sheep V over a period of 3 years during the earlier portion of which mixed infection was present. The imposed curve is based on Fig. 2 and represents the statistical range of the mean axial lengths plus and minus twice the standard deviation, this being computed to enclose 91.2% of the egg population.

COMPARISON OF EGGS DISSECTED FROM WORMS WITH THOSE OF FECAL ORIGIN

It is concluded that, as *H. contortus* found in sheep V at post mortem considerably outnumbered other species and because the egg-laying rate of the former species is known to exceed that of other trichostrongylids found in sheep, the measurements of eggs from feces obtained from this sheep at the time of autopsy (Fig. 3) may be regarded as differing insignificantly from what might have been derived from a pure infection.

The methods of sampling material for measurement differed between the two series. Eggs of fecal origin were the contributions of many worms which individually were represented in the total in proportion to their fecundity. On the other hand eggs dissected from worms were obtained from a small fraction of the entire population of parasites and further, the numbers measured were not numerically related to the potential egg-laying rate of the individuals dissected.

In regard to the worms used for dissection it is concluded, from the bivariate normal frequency distribution of the means of the axes of eggs from individuals (Fig. 7), that the samples adequately represented the general population of worms.

The similar coefficients of variation for fecal eggs from sheep V and for the aggregated results from dissections, respectively 4.0 per cent and 4.9 per cent for lengths and 3.8 per cent and 5.3 per cent for widths, were indicative that any error arising from disregarding the rate of egg-laying was insufficient to invalidate comparison of the two series of data. It follows that in computing means for dissected-out-eggs there was little difference between values derived from averaging the aggregated data, and the unweighted values from averaging the means for individual worms.

As shown in Table 1 means for the aggregated data for eggs from dissections were found to be 76.61 ± 0.09 μ by 41.25 ± 0.05 μ and those for fecal eggs from sheep V 78.49 ± 0.19 μ by 44.90 ± 0.11 μ. Thus it is seen that they reflect the relation between the two series of data shown in the range of fluctuation (Figs. 7 and 3), which involved almost complete overlapping of the long axes but, in regard to the short axes, a lesser degree of overlapping.

A small degree of negative or an absence of correlation was displayed between the long and the short axes (Figs. 3 and 7).

From the foregoing it is evident that the most marked difference between the two series of data was in connection with mean width. This is the dimension which would be expected to be most affected by any change in volume due to increase in internal pressure in eggs. However, what factors were operating between the time of laying of the eggs and the examination of the eggs under the microscope and were instrumental in

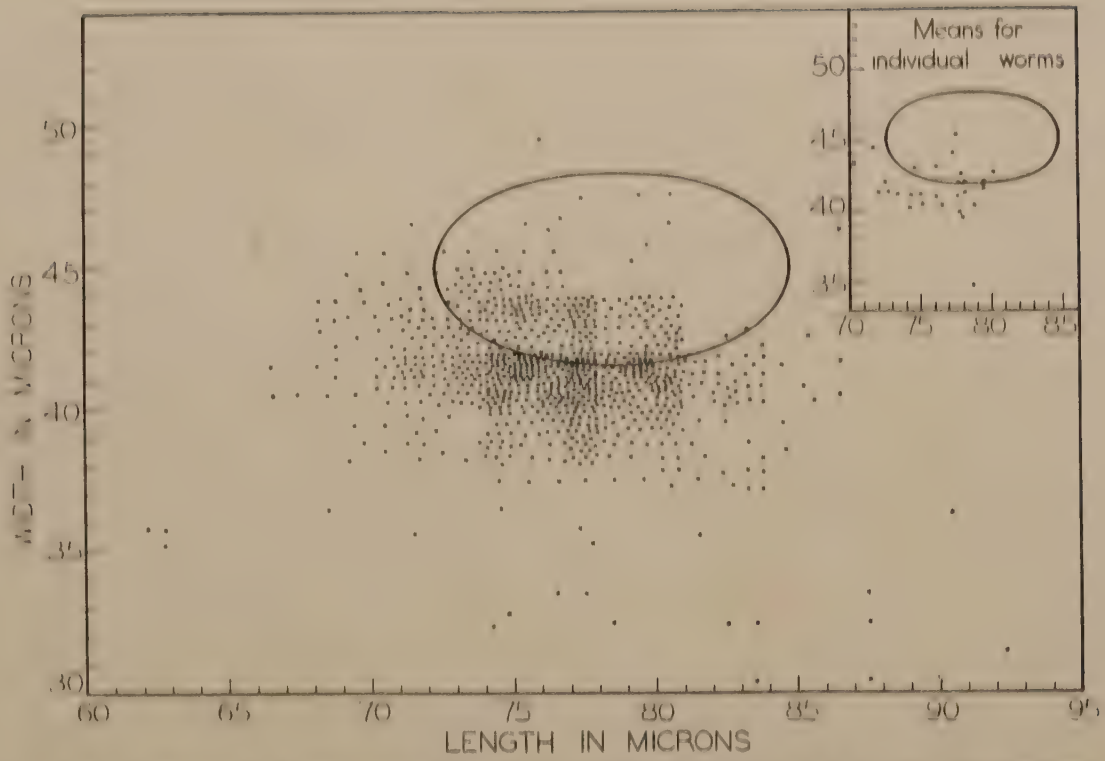

FIG. 7. Comparison of the measurements of eggs of *Haemonchus contortus* obtained by dissection with those from faeces, to demonstrate the greater diameter of the latter. Length has been plotted against the width of eggs dissected from worms from sheep I–V (see Table 1). The imposed curve is based on the measurements of eggs from the faeces of sheep V (see Table 1 and Fig. 3).

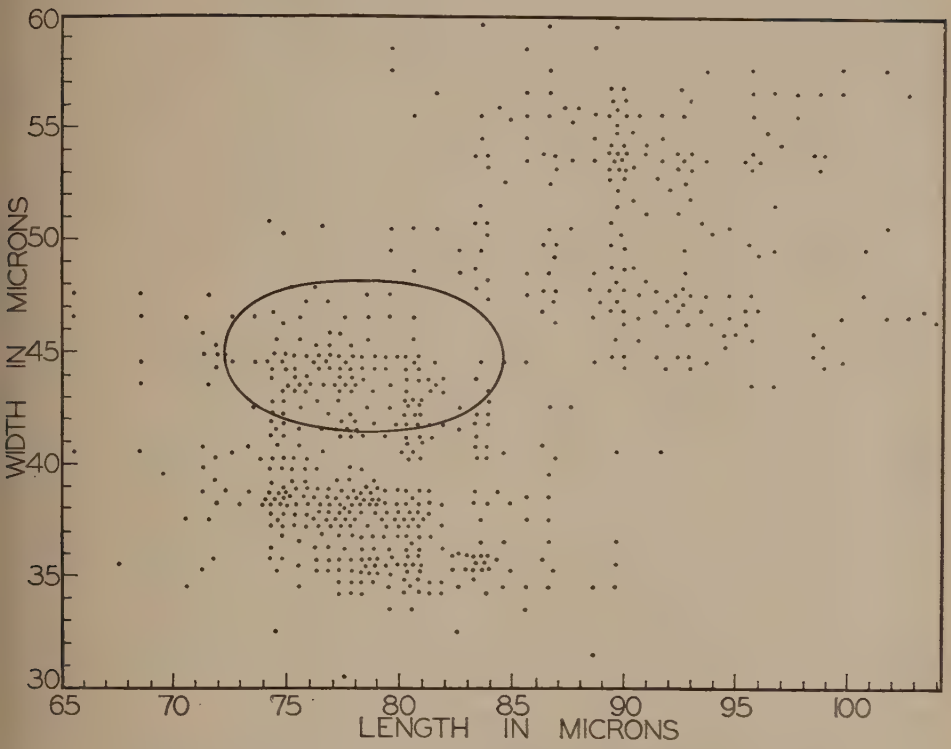

Fig. 8. Showing that a curve based on measurements of *Haemonchus contortus* eggs from the feces of sheep V and computed to enclose 91.2 per cent of the population of eggs fits a group of measurements of eggs from the feces of 50 mixed infections.

causing the change in volume are not indicated by the data. It is possible that the technical methods used were not entirely responsible.

Conclusions as to differences among the data of fecal origin from sheep V, VI and VII cannot be drawn. The latter two sheep, of different breed from sheep V, contained experimentally administered small infections of *H. contortus* and had been maintained indoors; further, another technique, centrifugal flotation, had been used in isolating the eggs from feces; then also there was the possible existence of strains among the parasites and of other factors associated with the geographically widely separated origin of the material; these all, conceivably, might have contributed causally to the differences.

APPLICATION OF RESULTS IN THE IDENTIFICATION OF EGGS VOIDED IN FECES

When plotted, the complete data relating to eggs of fecal origin from sheep V and from the flock of 50 sheep fell into groups between which there were various degrees of overlapping (Figs. 4 and 8). In each series a group coincided with that established for *H. contortus* from measurements made on eggs from feces of sheep V collected at the time of autopsy (Fig. 3). The conclusion is that, as the identity of the *H. contortus* grouping was well defined in data collected from the one sheep over a period of three years and from the flock of 50 sheep over several months, the frequency was characteristic of the species.

Applying to the complete data for sheep V (Fig. 4) and to that for the flock of 50 sheep (Fig. 8) a curve, constructed on the basis of measurements of fecal eggs from sheep V made at the time of autopsy and estimated to enclose 91.2 per cent of the population, it is seen that it encircles the core of the *Haemonchus* group in each frequency series. It is concluded that eggs mainly of *H. contortus* were found within the range of the curve.

In the presence of overlapping species it is considered that greater accuracy in estimating the number of *H. contortus* eggs would be achieved by applying curves containing smaller fractions of the population for instance 25 per cent (i.e., means plus and minus 0.6745 the standard deviation), as it is to be expected that towards the center of a distribution the proportional contamination by other species would be reduced. The measurement of large numbers of eggs is an obvious requirement for the successful application of the method.

It is possible that constants for *Haemonchus* will have to be determined for various conditions, inasmuch as host species or breed, strain of parasites and technical methods used might, to varying degrees, influence the form of the eggs; results from sheep VI and VII (Figs. 5 and 6) suggest this.

VALUE OF MEASUREMENTS OF EGGS DISSECTED FROM WORMS

The results plotted in Fig. 1 are designed to show that inter-specific differences in egg measurements of material from the one sheep were well marked; to this extent they supplement the findings of Shorb (1940) who does not specify the number of animals from which he obtained his data. It is reasonable to expect that parallel differences will exist in the dimensions of eggs found in feces and that, therefore, similar constants might be established for these other species on the lines given above for *H. contortus*. The dimensions of eggs dissected from females may possibly have an intrinsic value when treated statistically from the point of view

of their use in the specific identification of females in certain genera, at present distinguishable only with difficulty.

SUMMARY

Measurements of eggs of *Haemonchus contortus* were made in New Zealand, using material derived partly from dissections of females and partly from the feces of sheep. Standard techniques were used.

It was concluded that swelling of eggs took place during the interval between laying and the time of their examination under the microscope.

When lengths of eggs were plotted against widths the population had a bivariate normal frequency distribution.

The statistical range of means plus and minus twice the standard deviation (theoretically enclosing 91.2 per cent of the population) for eggs from feces treated by the Stoll technique was found to be 72–85 μ by 41–48 μ. This range fitted a group in data collected by the same technique from mixed infections in one sheep over three years and fifty sheep over several months.

In mixed infections where overlapping in dimensions by other species takes place it is suggested that the smaller range, 76–81 μ by 44–46 μ (means plus and minus 0.6745 the standard deviation and theoretically enclosing 25 per cent of the population), would enable the more accurate estimation of the numbers of *Haemonchus* eggs present. It is possible that the constants will be dependent on strain of parasites, technique used and host species or breed.

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RESEARCH NOTES

A CASE OF URINARY MYIASIS

A female patient, age 32, reported to Dr. S. J. Ohlhausen, of Houston, Texas, that she noticed about a dozen maggots in a toilet bowl after urination. Uncertain whether the maggots were actually voided with the urine or had gotten into the toilet bowl from some other source, she urinated further into a clean pan and found three more living maggots, which she brought to Dr. Ohlhausen, who, after killing and preserving them in formalin, referred them to the writer for identification. Examination showed them to be third-stage larvae of a *Lucilia*. The size and spacing of the posterior stigmal plates and the eight lobes of the anterior spiracles suggests that it is either *Lucilia sericata* or a closely related species.

The patient complained of pain in the bladder region and a burning sensation upon urination, which continued for several days after the worms were passed, although no more were found. The urinalysis on the day the worms were passed showed the following features: appearance, cloudy; reaction, alkaline; leucocytes, ++++; erythrocytes, ++; epithelial cells, occasional; albumin, ++++; and a heavy, mixed flora of motile bacilli. A urinalysis made two days after passage of the worms still showed abundant leucocytes and erythrocytes. Examination of the vulva and vagina showed no abnormal conditions. The patient offered no information which would indicate the source of the infection.—Asa C. Chandler, *Rice Institute, Houston, Texas.*

EXAMINATIONS OF WILD ANIMALS FOR THE CATTLE TICK *BOOPHILUS ANNULATUS MICROPLUS* (CAN.) IN FLORIDA

The recent attempt to free Florida of the North American cattle tick, *Boophilus annulatus* (Say), and its southern variety, *B. a. microplus* (Can.), by reducing the deer population in certain sections where this tick had not yielded to the usual eradication procedure, offered an opportunity to make a collection of wild hosts in the remaining "ticky" areas of the state. The majority of the collections were made during the months of November, December, January, and February, 1936–37, in collaboration with the U. S. Bureau of Animal Industry and the Florida State Livestock Board. A few records are included that were made in June, 1933, by O. C. Van Hyning. The collections were from Orange, Osceola, and Collier Counties, where the cattle ticks were known to occur. Since the host-parasite relationships are well established for domestic animals, the few records for such animals are included here only to give data on species other than cattle ticks. The records for wild birds and animals that follow include fairly representative numbers of the principal forms in the region. Although the number of hosts examined in this survey is not large, it is thought that the records should be published because of the present active interest in this question. These records are in accord with experimental data gathered by research units and with observations made by many men working on the tick problem. They sustain the conclusion that wild birds and animals other than deer do not serve as hosts for cattle fever ticks. In Table 1 are listed the 16 hosts with the 9 species of ticks collected. Following the table is a list of animals examined that harbored no ticks. The numbers in parentheses placed after the name of the hosts that were negative indicate the number of specimens examined.

Of the 22 deer examined (all from Orange County), 4, which were killed in a large cypress swamp, were infested with cattle ticks, the average infestation being 69, with a range from 3 to 182. The 18 deer on which there were no cattle ticks were taken from pine flat woods. This absence of cattle ticks on deer taken from the woods appears to be correlated with a program of ranging cattle over the tick-infested areas and dipping them at regular intervals. In the swampy areas it has not been possible to control ticks with such a cattle-dipping program, because the cattle do not move about freely in the dense swamps frequented by deer.

TABLE 1.—*Species of ticks collected from wild and domesticated animals in the cattle tick areas of Florida*

Hosts			Species of ticks								
	Number of specimens examined	Number Infested with ticks	*Haemaphysalis leporis palustris* Pack.	*H. chordeilis* Pack.	*Amblyomma maculatum* Koch	*A. americanum* (L.)	*Dermacentor variabilis* (Say)	*Ixodes texanus* Banks	*I. ricinis scapularis* Say	*Rhipicephalus sanguineus* (Latr.)	*Boophilus annulatus microplus* (Can.)
BIRDS											
Red-bellied woodpecker, *Centurus carolinus* (L.)	1	1			+						
Southern meadowlark, *Sturnella magna argutula* Bangs	55	42	+	+							
White-eyed towhee, *Pipilo erythrophthalmus alleni* Coues	1	1	+	+							
Florida grasshopper sparrow, *Ammodramus savannarum floridanus* (Mearns)	1	1			+						
MAMMALS											
Florida opossum, *Didelphis virginiana pigra* Bangs	4	3					+		+		
Florida raccoon, *Procyon lotor elucus* Bangs.	43	16					+	+	+		
Florida gray fox, *Urocyon cinereoargenteus floridanus* Rhoads	1	1			+				+		
Florida bobcat, *Lynx rufus floridanus* (Raf.)	2	2							+		
Florida cotton mouse, *Peromyscus gossypinus palmarius* Bangs	22	15					+				
Florida cotton rat, *Sigmodon hispidus littoralis* Chapman	25	21					+				
Florida cottontail, *Sylvilagus floridanus floridanus* (Allen)	1	1	+								
Florida marsh rabbit, *S. palustris paludicola* (Miller and Bangs)	4	3	+								
Florida deer, *Odocoileus osceola* (Bangs).	22	13			+				+		+
Hog	10	9			+		+		+		
Dog	9	4					+		+	+	
Man	?	3			+	+			+		

The following mammals were examined and found to have no ticks on them: Florida skunk, *Mephitis elongata* (Bangs) (3), Southern gray squirrel, *Sciurus carolinensis carolinensis* Gmelin (1), Southern fox squirrel, *S. niger niger* L. (2), and horse (4).

The following birds were examined and found to have no ticks on them: turkey vulture, *Cathartes aura septentrionalis* Wied. (2); Florida red-shouldered hawk, *Buteo lineatus alleni* Ridg. (2); Florida bob-white, *Colinus virginianus floridanus* (Coues) (2); Florida turkey, *Meleagris gallopavo osceola* Scott (2); killdeer, *Oxyechus vociferus vociferus* (L.) (1); Eastern mourning dove, *Zenaidura macroura carolinensis* (L.) (2); Florida barred owl, *Strix varia alleni* Ridg. (1); Florida burrowing owl, *Spcotyto cunicularia floridana* Ridg. (2); chuck-will's widow, *Antrostomus carolinensis* (Gmel.) (1); red-cockaded woodpecker, *Dryobates borealis* (Vieill.) (2); crow, *Corvus brachyrhynchos brachyrhynchos* Brehm (with some

doubt about the identity of the subspecies) (2); brown-headed nuthatch, *Sitta pusilla pusilla* Lath. (1); mockingbird, *Mimus polyglottos polyglottos* (L.) (1); robin, *Turdus migratorius migratorius* L. (with some doubt about the identity of the subspecies) (2); bluebird, *Sialia sialis grata* Banks (with some doubt about the identity of the subspecies) (3); loggerhead shrike, *Lanius ludovicianus ludovicianus* L. (3); and grackle, *Quiscalus quiscula aglaeus* Baird (with some doubt about the identity of the subspecies).

Identifications of the ticks were by F. C. Bishopp, C. N. Smith, and H. L. Trembley of the Bureau of Entomology and Plant Quarantine.—BERNARD V. TRAVIS, *Division of Insects Affecting Man and Animals, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.*

A COLLECTION OF OHIO TICKS AND THEIR HOSTS

A collection of ticks in Ohio by the writer, assisted by Dr. Floyd B. Chapman, yielded the following information. There are forty records, including six records of retrapped animals. The hosts were seven mammalian species, three avian species, and four records of ticks collected from vegetation by a flagging method. Collection was made during September, October, and November in 1936, and April through October in 1937. Three genera of ticks are recognized, including five species: *Haemaphysalis leporis-palustris, Dermacentor variabilis, Ixodes cookei, Ixodes diversifossus, Ixodes marxi.*

I. *Haemaphysalis leporis-palustris.*
  A. Nile Twp., Scioto Co.
    1. Hosts—22 rabbits (*Sylvilagus floridanus mearnsi*)
      (a) adults: May 5, June 28, 30, July 5, 10, 11, 12, ?, 13—all in 1937.
      (b) nymphs: Sept. 24, 27, Oct. 4, 6, 26, Nov. 21 in 1936; Aug. 2, 1937.
      (c) larvae: Sept. 27, Oct. 6, 1936; Aug. 7, 1937.
    2. Host—1 catbird (*Dumetella carolinensis*)
      (a) adult: May 5, 1937.
    3. Host—1 quail (*Colinus virginianus virginianus*)
      (a) nymph: Oct. 26, 1936.
  B. Oxford Twp., Erie Co.
    1. Hosts—4 rabbits (*Sylvilagus floridanus mearnsi*)
      (a) adults: July ?, 1937.
      (b) nymphs: Aug. 3 and 25, 1937.
      (c) larvae: Aug. 25, Sept. 21, 1937.
    2. Host—2 records collected from vegetation.
      (a) larvae: Aug. 20 and 28, 1937.
  C. Huron Co. (northern part).
    1. Host—1 starling (*Sturnus vulgaris vulgaris*)
      (a) larva: Aug. 10, 1937.
  D. Licking Co. (Linville, O.).
    1. Host—1 rabbit (*Sylvilagus floridanus mearnsi*)
      (a) nymphs: Sept. 18, 1937.

II. *Dermacentor variabilis.*
  A. Nile Twp., Scioto Co.
    1. Host—1 man
      (a) adult: 1 record between July and August, 1937.

III. *Ixodes cookei.*
  A. Oxford Twp., Erie Co.
    1. Hosts—2 woodchucks (*Marmota monax*)
      (a) adults: Aug. 19, 28 in 1937.
      (b) nymphs: Aug. 19, 28 in 1937.
      (c) larvae: Aug. 19, 1937.
    2. Host—1 opossum (*Didelphis virginiana*)
      (a) nymphs: Sept. 4, 1937.

3. Host—1 weasel (*Mustela noveboracensis*)
   (a) adults, nymphs, and larvae: all on Sept. 20, 1937.
4. Host—1 record collected from vegetation
   (a) nymph: Aug. 3, 1937 (identification not definitely settled).

IV. *Ixodes diversifossus.*
   A. Nile Twp., Scioto Co.
      1. Hosts—3 rabbits (*Sylvilagus floridanus mearnsi*)
         (a) adults: Apr. 27, July 5 and 10, 1937.
   B. Oxford Twp., Erie Co.
      1. Host—1 rabbit (*Sylvilagus floridanus mearnsi*)
         (a) larvae: Aug. 25, 1937.
      2. Host—1 record collected from vegetation.
         (a) nymph: July ?, 1937 (identification not definitely settled).

V. *Ixodes marxi.*
   A. Nile Twp., Scioto Co.
      1. Host—1 gray squirrel (*Sciurus carolinensis carolinensis*)
         (a) adult: Oct. 3, 1936.
   B. Ashtabula Co.
      1. Host—1 red squirrel (*Sciurus hudsonicus*)
         (a) adult: Sept. 16, 1937.

Haemaphysalis leporis-palustris and *I. diversifossus* were found associated on two rabbits in Scioto Co., and one rabbit in Erie Co. *I. cookei* was recovered in all three developmental stages from one woodchuck and one weasel in Erie Co.

This work was performed while a research fellow under Dr. L. E. Hicks at the Ohio Wildlife Research Station, Columbus, Ohio, 1936–1938. Tick species were determined by Dr. F. C. Bishopp and Miss H. L. Trembley of the Department of Agriculture, Washington, D. C.—Julius S. Katz, *Junior Veterinarian, U. S. Bureau of Animal Industry.*

PTERODRILUS ALCICORNUS IN VIRGINIA

In 1895 Moore described a curious branchiobdellid from John's River, Watauga Co., North Carolina. This species he named *Pterodrilus alcicornus* and made it the type of his new genus, *Pterodrilus.* He named the species *alcicornus* from the dorsal appendages, "Their shape being strongly suggestive of the antlers of a young moose."

Since this original specimen described by Moore, no more have been reported. Although the writer had extensive collections from the Eastern mountains during a recent survey of this group, no individual of this species was encountered; so it had to be considered a rare species known only from the type material.

Recently in a collection of worms from Giles Co., Virginia, the writer was astonished to see two specimens of *Pterodrilus alcicornus.* These agreed in every way with Moore's original descriptions. The specimens were sent by Dr. Horton H. Hobbs, Jr., of the University of Florida. They were taken from the crayfish, *Cambarus bartoni,* on August 13, 1940. The infected crayfish were collected in a rocky pool of a small mountain creek known locally as Sinking Creek near Mt. Lake, Giles Co., Virginia.

Giles Co., Virginia, is about 120 miles north of the type locality, and is in the same type of country. *Pterodrilus alcicornus* is probably a species of a very local distribution confined to a small area of the Eastern mountains. While many species of branchiobdellids are wide ranging, some, especially mountain forms of the genus *Pterodrilus,* may be very restricted. This is, of course, the first record of this branchiobdellid from the state of Virginia.—Clarence J. Goodnight, *Biology Department, Brooklyn College.*

IN MEMORIAM

Adolpho Lutz (1855–1940)

The death of Dr. Adolpho Lutz on October 6, 1940, terminated the career of one of the ablest and most distinguished scientists of the past generation. He was born in 1855 at Rio de Janeiro, of Swiss parents who came to Brazil in 1849. His father's family had lived in Berne for more than five hundred years and his mother's family resided in a neighboring canton. Adolpho Lutz was taken to Switzerland at the age of two and educated there. He graduated in medicine at the University of Berne in 1879 and was interne at the Hôpital de St. Gallen. He continued his training at Leipzig (with Leuckart), Prague, Vienna, London (with Lister), and Paris (with Pasteur).

With this admirable preparation, he returned to Brazil in 1881 and began the practice of medicine in the interior of the state of São Paulo. His training with the European masters, however, had stimulated a deep interest in research and his work as a physician was augmented by investigations of hookworm and other parasites, chiefly helminths. In 1888 he returned to Europe, where he worked in the dermatological institute of Prof. Unna in Hamburg. He was then invited to direct the work on leprosy in Hawaii (1889–1891) where he met and married an Englishwoman, Amy Fowler, who had gone there as a volunteer nurse for the lepers. From Hawaii, Dr. Lutz went to California where he secured a license to practice medicine. But he soon (1892) returned to Brazil where he reestablished practice and became a member of the staff of the newly organized Bacteriological Institute of São Paulo. Professor Le Dantec had been brought from Paris as the first Director, but his French associate died of yellow fever and Le Dantec was so disturbed and distressed that he resigned his position, recommended the appointment of Dr. Lutz as his successor, and returned to France. Dr. Lutz served as Director of the Bacteriological Institute until September, 1908, when at the invitation of Oswaldo Cruz he went to Rio as Director of the Section of Medical Zoology in the Instituto de Manguinhos, later the Instituto Oswaldo Cruz, which offered more time and opportunity for investigation. He had become more interested in research than in medical practice and found administrative routine an irksome task. He remained at the Instituto Oswaldo Cruz until 1938, although he had been officially retired two years earlier, at the age of eighty-one, when a regulation was adopted retiring all civil servants more than sixty-eight years old.

Like other physicians who were also great parasitologists, notably Leidy, Leuckart and Fülleborn, the principal factor in Adolpho Lutz's

work and career is to be found in his devotion to natural history. His absorbing interest in fundamental biological phenomena was the wellspring from which arose the extensive list of his discoveries in biology and medicine. As a boy of five, he was studying animals and plants. His first paper, published in 1878 and awarded a prize by the naturforschenden Gesellschaft in Berne, was on the Cladocera of the Bernese region. On his first return to Brazil he began his researches in parasitology, especially on hookworm, other endoparasites, and the blood-sucking Diptera. These investigations were continued on his subsequent return and in addition he extended the work to include the bacteria, fungi which attack the skin, and the parasitic protozoa. Endowed with an inquiring mind, he turned his attention to all kinds of disease-producing organisms. His patience and thoroughness are apparent in the results. Each new discovery was the starting-point for further investigation. His studies on schistosomiasis led in 1921 to the disclosure that certain forktailed cercariae undergo metamorphosis in intermediate hosts to produce the tetracotyliform larvae, thus correcting the idea of Leuckart that strigeid trematodes have a "metastatic" type of development. This discovery was confirmed almost immediately by Mathias in France and Szidat in Germany, and has provided the basis for the great advance in knowledge of the furcocercous cerceriae and the STRIGEIDAE. His investigations of trematode life histories were supplemented by studies on the snails which serve as intermediate hosts. His work on the parasites of amphibians led to the beautifully illustrated monograph on the batrachians of Brazil. From his early years he conducted researches on the blood-sucking arthropods, which culminated in many shorter articles and monographs of several families of Diptera. Familiar with the life histories, habits and distribution of the Culicidae of Brazil, he was able to indict the *Aëdes* (*Stegomyia*) as the transmitting agent of yellow fever and he instituted the antimosquito campaign in São Paulo immediately after the results of the Havana experiment were communicated to him by letter. To confirm the work, he raised mosquitos and repeated the experiment on himself. In recognition, the state of São Paulo struck a gold medal in his honor. He published on the blood-inhabiting Sporozoa of lower vertebrates, Microsporidia, trypanosome infection in cattle, and a host of other subjects, in addition to the reports of the laboratory of which he was the Director. The study of parasites and parasitic infections in lower animals afforded the primary information for his discoveries in human medicine.

Dr. Lutz was an enthusiastic collector. He assembled some 3000 herbarium specimens in addition to his extensive helminthological, entomological and herpetological collections. These specimens, together with his letters and other papers, are housed in the Instituto Adolpho Lutz at São Paulo, dedicated three weeks after his death.

The medical contributions of Dr. Lutz are too extensive and too well known to require enumeration here. He introduced vaccination in South America, instituted isolation and quarantine against smallpox, typhoid and yellow fever, and through his vigorous public health measures eliminated yellow fever, bubonic plague and cholera from São Paulo. His more important achievements include: (1) the identification of the "febres paulistas" of São Paulo as typhoid, a determination which was confirmed on cultures by the Institut Pasteur and on anatomical findings by Eberth; (2) the discovery before 1903 of epidemics of yellow fever in forested areas where he knew there were no "*Stegomyia,*" but other closely related species; (3) the demonstration (1903) of the transmission of malaria by jungle mosquitos, *Anopheles lutzi* Theobald, which breed in the leaf cups of bromeliad plants; (4) the discovery of granulation in the acid-fast bacilli of leprosy and the proposal (1886) of the name *Coccothrix leprae* for this organism. The study of leprosy for more than fifty years, and especially consideration of its epidemiology, persuaded him that the disease could be transmitted by mosquitos.

Dr. Lutz traveled widely in Europe and the Americas, and was a member of many scientific societies. An accomplished linguist, he was an official delegate on many scientific and medical missions and received numerous medals and decorations from South American and European governments. Brief accounts of his work were published in North America on the occasion of his seventieth birthday by Metcalf (1926, Scientific Monthly, 22: 112–114) and after his death by Helen Gaige (1940, Copeia, No. 4, pp. 275–276). The Memorias do Instituto Oswaldo Cruz, Vol. 18 (1925) contains an intimate article by Dr. Carlos Chagas and a bibliography of his published work. A complete bibliography, prepared by Dr. Neiva and containing over 200 titles, is to be published in the forthcoming number from the Instituto Oswaldo Cruz.

Adolpho Lutz is survived by a sister, now in her ninety-second year, a son, Dr. Gualter Adolpho Lutz, Professor of Forensic Medicine at the University of Brazil, and a daughter, Bertha Lutz, Chief Naturalist of the National Museum at Rio de Janeiro. His wife died in 1922. To his daughter, who had been his companion and associate in the investigations of his later years, I am indebted for much of the material in this article.

Dr. Lutz was a modest and gracious person. His long life of service and accomplishment was crowned with the highest honors, the affection of a host of friends, and the esteem of scientists and medical men everywhere.—Horace W. Stunkard, *New York University.*

AMERICAN SOCIETY OF PARASITOLOGISTS

PRELIMINARY ANNOUNCEMENT OF THE SEVENTEENTH ANNUAL MEETING

MONDAY, TUESDAY AND WEDNESDAY, DECEMBER 29–31, 1941

DALLAS, TEXAS

In accordance with the decision of the Council, acting under authority voted at the sixteenth annual business meeting in Philadelphia, the American Society of Parasitologists will convene for a three-day program in conjunction with the meeting of the American Association for the Advancement of Science in Dallas, Texas. Hotel Adolphus has been designated as official headquarters for the Society.

Sessions for the reading of contributed papers are scheduled on the first and third days. The main features of the program are arranged for the second day, Tuesday, December 30th. At the conclusion of the morning session, Dr. James E. Ackert will deliver the presidential address on the subject "Natural Resistance to Helminthic Infections." The annual Parasitologists' Luncheon will be held Tuesday noon and will be followed by the annual business meeting of the Society. In the afternoon, the demonstration program and tea will be held in the laboratories of Southern Methodist University.

The call for papers has already been mailed to members of the Society. In preparing abstracts, members are requested to consult the styling used in the December, 1940, Supplement of the Journal in order that editing may be kept at a minimum. Members are reminded that all abstracts for the program must be in the office of the Secretary not later than Friday, October 24, 1941.

O. R. McCOY. *Secretary*